Studies in Natural Products Chemistry

Atta-ur-Rahman, FRS
Editor

Volume 37

Studies in Natural Products Chemistry

Volume 37

Bioactive Natural Products

Edited by

Atta-ur-Rahman, FRS
International Center for Chemical and Biological Sciences,
H.E.J. Research Institute of Chemistry,
University of Karachi,
Karachi 75270, Pakistan

Amsterdam • Boston • Heidelberg • London • New York • Oxford
Paris • San Diego • San Francisco • Singapore • Sydney • Tokyo

Elsevier
The Boulevard, Langford lane, Kidlington, Oxford, OX5 1GB, UK
Radarweg 29, PO Box 211, 1000 AE Amsterdam, The Netherlands

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

ISBN: 978-0-444-59514-0
ISSN: 1572-5995

Contributors

Pérez-Alvarez José Angel
IPOA Research Group (UMH-1 and REVIV-Generalitat Valenciana). AgroFood Technology Department. Escuela Politécnica Superior de Orihuela. Miguel Hernández University. Crta. Beniel km. 3.2. E-03312 Orihuela Alicante, Spain

Ammar Bader
Department of Pharmacognosy, Faculty of Pharmacy, Umm Al-Qura University, P.O. Box 13174, 21955 Makkah, Saudi Arabia

Alessandra Braca
Dipartimento di Scienze Farmaceutiche, Università di Pisa, Via Bonanno 33, 56126, Pisa, Italy

Maurício Da Silva Brandão
Instituto de Química, Universidade Federal da Bahia, 40170-290, Salvador – BA, Brazil

Paulina Bermejo Benito
Department of Pharmacology, Faculty of Pharmacy, University Complutense, Ciudad Universitaria s/n, 28040, Madrid, Spain

Heloísa Helena Garcia Da Silva
Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, CEP 74001-970 Goiânia, GO, Brazil

Ionizete Garcia Da Silva
Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, CEP 74001-970 Goiânia, GO, Brazil

Mariana Galata
University of British Columbia, Okanagan campus, Biology, SCI Building, 3333 University Way, Kelowna, BC V1V 1V7, Canada

Francesca R. Gallo
Department of Therapeutic Research and Medicines Evaluation, Istituto Superiore di Sanità, V.le Regina Elena 299I-00161, Rome, Italy

Regina Geris
Instituto de Química, Universidade Federal da Bahia, 40170-290, Salvador – BA, Brazil

Ahmad Reza Gohari
Medicinal Plants Research Center, Tehran University of Medical Sciences, Tehran, PO Box 14155-6451, Iran

Robert A. Halberstein
Department of Anthropology and School of Nursing and Health Studies University of Miami, P.O. Box 248106, Coral Gables, Florida 33124-2005, USA

Elena Federici
Department of Therapeutic Research and Medicines Evaluation, Istituto Superiore di Sanità, V.le Regina Elena 299I-00161, Rome, Italy

Warjeet s. Laitonjam
Department of Chemistry, Manipur University, Imphal-795003, Manipur, India

Fernández-López Juana
IPOA Research Group (UMH-1 and REVIV-Generalitat Valenciana). AgroFood Technology Department. Escuela Politécnica Superior de Orihuela. Miguel Hernández University. Crta. Beniel km. 3.2. E-03312 Orihuela Alicante, Spain

Chao Luo
Department of Plant Pathology, College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, China

Soheil Mahmoud
University of British Columbia, Okanagan campus, Biology, SCI Building, 3333 University Way, Kelowna, BC V1V 1V7, Canada

Viuda-Martos Manuel
IPOA Research Group (UMH-1 and REVIV-Generalitat Valenciana). AgroFood Technology Department. Escuela Politécnica Superior de Orihuela. Miguel Hernández University. Crta. Beniel km. 3,2. E-03312 Orihuela Alicante, Spain

María José Abad Martínez
Department of Pharmacology, Faculty of Pharmacy, University Complutense, Ciudad Universitaria s/n, 28040, Madrid, Spain

Giuseppina Multari
Department of Therapeutic Research and Medicines Evaluation, Istituto Superiore di Sanità, V.le Regina Elena 299I-00161, Rome, Italy

Marcello Nicoletti
Department of Enviromental Biology, University Sapienza of Rome, P.le A. Moro 5I-00185, Rome, Italy

Luis Miguel Bedoya Del Olmo
Department of Pharmacology, Faculty of Pharmacy, University Complutense, Ciudad Universitaria s/n, 28040, Madrid, Spain

Giovanna Palazzino
Department of Therapeutic Research and Medicines Evaluation, Istituto Superiore di Sanità, V.le Regina Elena 299I-00161, Rome, Italy

Valentina Petitto
Department of Enviromental Biology, University Sapienza of Rome, P.le A. Moro 5I-00185, Rome, Italy

Paulo Roberto Ribeiro
Instituto de Química, Universidade Federal da Bahia, 40170-290, Salvador – BA, Brazil

Soodabeh Saeidnia
Medicinal Plants Research Center, Tehran University of Medical Sciences, Tehran, PO Box 14155-6451, Iran

Tijiang Shan
Department of Plant Pathology, College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, China

Luis Apaza Ticona
Department of Pharmacology, Faculty of Pharmacy, University Complutense, Ciudad Universitaria s/n, 28040, Madrid, Spain

Nunziatina De Tommasi
Dipartimento di Scienze Farmaceutiche e Biomediche,Università di Salerno, Via Ponte Don Melillo, 84084 Fisciano, Salerno, Italy

Jingguo Wang
Department of Plant Nutrition, College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China

Kui Wang
Department of Plant Pathology, College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, China

Jianmei Xu
Department of Plant Pathology, College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, China

Jianglin Zhao
Department of Plant Pathology, College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, China

Ligang Zhou
Department of Plant Pathology, College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, China

Preface

The present volume, representing volume 37 of this longstanding series, contains a number of interesting articles covering a diversity of natural products. The article by Halberstein describes botanical medicines used in diuresis in traditional medicinal systems. The chemistry, biological activity, mechanisms of action and structure-activity relationships of bioactive sesquiterpene lactones in genus *Artemisia* are reviewed by Martinez and coworkers. A large number of metabolites are involved in plant-nematode interactions. These include repellents, attractants, hatching stimulants or inhibitors, and nematotoxicants. Zhou and coworkers have discussed such plant-derived antinematodal secondary metabolites. The beneficial effects on human health of bioactive compounds in spices and herbs have been reviewed by Juana et al. Biosynthetic and biotechnological aspects of plant isoprenoids are presented by Galata and Mahmoud. Problems associated with therapy of the patients with against African and American trypanosomiasis include frequent side-effects and human trypanosomiasis. These "poor man's diseases" afflict people in Africa or Latin America, and little investment is made by the pharmaceutical industry in drug development in these fields. Trypanocidal monoterpenes that can serve as lead compounds for future trypanocidal drugs are discussed in the review by Saerdnia and Gohari. The structures, biological activities and taxonomic relationships of 3, 4-dihydroisocoumarins from plants and fungi are reviewed by Braca and coworkers. The use of HPTLC approach to identify natural products and botanicals is presented by Nicoletti and Petitto. Plant anti-oxidants can serve as natural free radical scavengers. Antioxidant activity can counter act the strongly oxidizing environment in which we live and offer protection from mutagenesis, carcinogenesis and aging. This area has been reviewed by Laitonjam. The mosquito *Aedes aegypti* is the vector responsible for dengue and yellow fever. Bioactive secondary metabolites produced by plants, fungi, bacteria (actinomycetes and cyanobacteria) and lichens which have activity against the mosquito *Aedes aegypti* are discussed by Geris and coworkers.

This volume with its emphasis on bioactive natural products against many neglected diseases should prove to be of great interest to scientists working in the field of medicinal and natural product chemistry.

I would like to express my sincere thanks to Mr. Mahmood Alam for his support in the preparation of this volume. I would also like to thank Miss Taqdees Malik and Mr. Wasim Ahmad for secretarial assistance.

PROF. ATTA-UR-RAHMAN, *FRS*
Patron-in-Chief,
International Centre for Chemical & Biological Sciences,
University of Karachi,
Karachi, Pakistan

STUDIES IN NATURAL PRODUCTS CHEMISTRY

Edited by Atta-ur-Rahman

Vol. 1 Stereoselective Synthesis (Part A)
Vol. 2 Structure Elucidation (Part A)
Vol. 3 Stereoselective Synthesis (Part B)
Vol. 4 Stereoselective Synthesis (Part C)
Vol. 5 Structure Elucidation (Part B)
Vol. 6 Stereoselective Synthesis (Part D)
Vol. 7 Structure and Chemistry (Part A)
Vol. 8 Stereoselective Synthesis (Part E)
Vol. 9 Structure and Chemistry (Part B)
Vol. 10 Stereoselective Synthesis (Part F)
Vol. 11 Stereoselective Synthesis (Part G)
Vol. 12 Stereoselective Synthesis (Part H)
Vol. 13 Bioactive Natural Products (Part A)
Vol. 14 Stereoselective Synthesis (Part I)
Vol. 15 Structure and Chemistry (Part C)
Vol. 16 Stereoselective Synthesis (Part J)
Vol. 17 Structure and Chemistry (Part D)
Vol. 18 Stereoselective Synthesis (Part K)
Vol. 19 Structure and Chemistry (Part E)
Vol. 20 Structure and Chemistry (Part F)
Vol. 21 Bioactive Natural Products (Part B)
Vol. 22 Bioactive Natural Products (Part C)
Vol. 23 Bioactive Natural Products (Part D)
Vol. 24 Bioactive Natural Products (Part E)
Vol. 25 Bioactive Natural Products (Part F)
Vol. 26 Bioactive Natural Products (Part G)
Vol. 27 Bioactive Natural Products (Part H)
Vol. 28 Bioactive Natural Products (Part I)
Vol. 29 Bioactive Natural Products (Part J)
Vol. 30 Bioactive Natural Products (Part K)
Vol. 31 Studies in Natural Products Chemistry:
Cumulative Indices Vol. 1-30
Vol. 32 Bioactive Natural Products (Part L)
Vol. 33 Bioactive Natural Products (Part M)
Vol. 34 Bioactive Natural Products (Part N)
Vol. 35 Bioactive Natural Products (Part O)
Vol. 36 Bioactive Natural Products
Vol. 37 Bioactive Natural Products

Chapter 1

Botanical Medicines for Diuresis: Cross-Cultural Comparisons

Robert A. Halberstein
Department of Anthropology and School of Nursing and Health Studies, University of Miami, P.O. Box 248106, Coral Gables, USA

INTRODUCTION

Modern synthetic drugs have a remarkably recent history. Prior to the beginning of the twentieth century most medications were derived directly from natural plant, animal, or mineral sources. Archaeological evidence indicates that medicinal plants have been exploited for a variety of healing purposes for at least 15,000 years [1–3]. It was discovered early on in those experiments that many plants are poisonous and boiling the collected specimens into a tea with various natural additives ("decoction") could enhance and speed their healing action by concentrating their beneficial phytochemicals while simultaneously eliminating toxins. Gradually, a wide range of propagation, preparation and application methods was developed to maximize the medicinal benefits and minimize the potentially deleterious effects of plant constituents. Consequently, botanical medicaments are used today cross-culturally in a great variety of forms—raw herb eaten or applied topically without modification, boiled liquid drink/tea, tablet, capsule, cream, extract, tincture, syrup, oil, salve, ointment, snuff, lozenge, plaster, poultice, powder, balm, emollient, paste, lubricant, inhalant, suppository, oral or nasal, juice, etc. [4].

One of the earliest purposes of ancient medicines was to eliminate disease-producing outside agents and harmful internal entities from the patient's body. Before the widespread use of the microscope these ominous invading forces were often considered to be of supernatural origin. A wide range of invisible culprits continue to be recognized as responsible for the incidence of diseases in many traditional cultures *via* "spirit possession" [5–7]. Many medicines and associated methods of administration have thus been produced

Bioactive Natural Products (Part Q), Vol. 37. http://dx.doi.org/10.1016/B978-0-444-59514-0.00001-8

in order to remove the irritants. Blood-letting is a practise dating to prehistoric times that is performed to purify the body by discharging poisons, evil spirits, and other harmful materials. Similarly, purging (inducing vomiting), sweat induction, diuresis and the use of laxatives and expectorants have all been prescribed by healers cross-culturally to help cleanse the patient's body and restore his/her natural equilibrium [8–10].

The purpose of the present paper is to compile and analyze the many botanical medicines that have been used cross-culturally to induce diuresis, or increased urine flow. Diuretic plants have held a central place in numerous traditional medical systems as a vehicle to expel poisons and an assortment of other disease-producing factors.

METHODS

Field work was undertaken at 92 "*botanicas*" (medicinal plant shops) in the metropolitan area of Miami, Florida. Surveys were completed at each establishment on the availability, preparation methods, and administration protocols of a variety of botanical remedies. Medicinal plant formulae were recorded that were recommended for the following chronic health conditions: high and low blood pressure, fertility-related disorders, diabetes, insomnia, and dental problems. Plant species prescribed as diuretics were also noted. The proprietors of 10 of the *botanicas* were interviewed in depth regarding herbal remedies and their combinations, recommended intake schedules, possible side effects, and sales trends. They also provided details about other health-related products available in the stores, including charms, amulets, religious articles (e.g., stylized crucifixes) and related literature, statuettes, animal effigies, mixing bowls and storage containers, perfumed candles and incense, jewellery, artworks, air fresheners, wind chimes, and a multipurpose bottled liquid medicinal formula called *Jarabe de Oro* ("Golden Tonic") that contains royal jelly, honey, bee pollen, vitamin B and extracts from selected anatomical parts of seven plant species.

Nine of the 10 informants from the *botanicas* stated they had received formal training in medicinal plant usage from established healers and herbalists in the Miami area. Each of the interviewees indicated they regularly consult published sources to update their knowledge of plant formulae and applications. Nearly a quarter of the *botanicas* were open 24h for sales, consultations, emergency treatments, and referrals to more specialized local healers or clinics and hospitals.

FINDINGS

Diuretics comprise a class of chemical agents which initiate, enhance and/or promote the loss of fluids from the body by triggering an increase in the rate of urine production and expulsion by the kidneys. Traditional and modern

healers have long recognized that edema, the accumulation of fluids in soft tissues, is a reliable and consistent symptom of disorders of the kidneys, spleen, liver, lungs, heart, blood vessels, and other elements of the genitourinary and circulatory systems [11]. Herbal remedies for edema were probably one of the earliest biomedical applications of natural diuretics. Hoffmann [12] classified the ingestible herbs with diuretic action into two broad categories: (1) plants which cause an increase in kidney blood flow and subsequent urine production, and (2) plants which inhibit or block water re-absorption by kidney nephrons.

Several corollary beneficial results of the application of diuretic phytochemicals have been recorded: anti-inflammatory, antimicrobial, antilithic (inhibiting renal system stone formation), astringent (producing tissue contraction or shrinkage), and demulcent (soothing or pain-reducing) effects. Yarnell *et al*. [13, p. 231] state that " Diuretic herbs can be added to any program for preventing kidney stone recurrence. They are safe and extremely cheap, even compared to the main diuretic drugs recommended for stone prevention, thiazide diuretics." The latter authors describe phytomedicinal formulae prescribed and administered to prevent renal calculi (stones) or to aid in their dissolution or expulsion. They tabulate seven plant species whose products in tincture form (e.g., ethanol extracts) can serve both purposes, albeit when combined in different proportions: *Agropyron repens, Equisetum arvense, Eupatorium purpureum, Hydrangea arborescens, Rubia tinctoria, Serenoa serrulata*, and *Taraxacum officinale*. The juice of the cranberry fruit (*Vacinium macrocarpon*) has a lengthy history of cross-cultural use in ethnomedicine as a urinary tract antiseptic cleanser and diuretic, although mixed results have been reported in controlled experimental trials [13].

A total of 406 botanical species with diuretic effects, including 13 sold in the Miami *botanicas* and an additional 393 recorded in published scientific field studies and textbooks, are presented in Table 1. Leaves and roots are the two most commonly used plant parts for this purpose. The entire compacted plant is utilized in the preparation methods of a large number of species. Boiling the whole plant or selected parts in water, fruit or vegetable juice, or another liquid solvent entails two different, but related, procedures. "Decoction" is the prolonged and thorough boiling of harder herbal components such as barks, branches, nuts, seeds, uncut roots, or the whole compressed plant. "Infusion" involves pouring boiling water/juice over finely cut or pulverized softer and more soluble plant components such as dried leaves, stems, bulbs, flowers, fruits, resins, or chopped roots. Both methods include simmering and straining the resulting processed mixtures. Unlike decoctions, infusions may be ingested cold or iced [4]. Additional phytomedical preparation and application methods are exhibited in Table 1, including ingestion of toasted nuts; smoking of dried leaves in a pipe or cigarette; ingestion of powdered leaf tablets; calyx infusion; and decoctions of gums, saps, tubers, berry powders, spores, rhizomes, rinds, seeds, stems and flowers. An additional compilation of diuretic plant species has recently been published [13a].

TABLE 1 Medicinal Plants Used as Diuretics

Species	Preparation	Reference
Abutilon indicum	Bark and root decoction	[32]
Acalypha arvensis	Flower and leaf decoction	[33, p. 134]
Acalypha indica	Plant decoction	[23, p. 395]
Acanthus spinosus	Plant decoction	[34, p. 515]
Achillea millefolium	Plant decoction	[17]
Achyranthes aspera		[32]
Achyranthes bidentata	Root decoction	[23, p. 395]
Adonis vernalis	Powdered flower decoction	[23, p. 353]
Aerva javanica	Plant/flower/root decoctions	[23, p. 396]
Aerva lanata	Leaf/stem infusions	[32]; This study
Aesculus hippocastanum	Roasted nut ingestion; bark decoction	[35, p. 34]
Agathosma betulina	Leaf decoction	[23, p. 35]
Agrimonia eupatoria	Plant decoction	[23, p. 36]
Agropyron repens	Root-stock/tuber decoctions	[36, p. 179]
Alcea rosea		[37, p. 54]
Alchemilla arvensis	Plant decoction	[34, p. 515]
Alchemilla xanthochlora	Leaf/shoot decoctions	[38]
Alisma plantago-aquatica	Root decoction	[23, p. 397]
Allium ampeloprasum	Raw plant ingestion/ decoction	[39, p. 266]
Allium cepa	Bulb decoction	[17]
Allium sativum	Bulb decoction	[37, p. 52]
Alternathera achyrantha	Stem and leaf infusions	[40]
Amaranthus dubius	Plant decoction	[23, p. 398]
Amaranthus hypochondriacus	Plant decoction	[23, p. 398]
Ammi majus	Fruit decoction	[34, p. 515]
Ammi visnaga	Fruit decoction	[34, p. 515]
Amomum thyrsoideum	Root decoction	[41]

TABLE 1 Medicinal Plants Used as Diuretics—Cont'd

Species	Preparation	Reference
Anagallis arvensis	Plant decoction	[23, p. 398]
Anemone pulsatilla	Plant decoction	[34, p. 515]
Anethum graveolens	Fruit decoction	[34, p. 47]
Annona squamosa	Dry leaves smoked	[42]
Angelica archangelica	Root/fruit//leaf/seed decoctions	[38]
Anthocleista nobilis	Seed/bark decoctions	[43, p. 109]
Anthriscus cerefolium	Plant decoction	[23, p. 399]
Aphanes arvensis	Plant decoction	[23, p. 399]
Apium graveolens	Plant/fruit/root/seed decoctions	[23, p. 47]
Apocynum androsaemilfolium	Root infusion	[23, p. 399]
Apocynum cannabinum	Root decoction	[23, p. 399]
Aralia racemosa	Root-stock/root decoctions	[36, p. 182]
Arctium lappa	Root decoction	[36, p. 173]
Arctium minus	Root/leaf decoctions	[44, p. 322]
Arctostaphylos tomentosa	Plant infusion	[45, p. 120]
Arctostaphylos uva-ursi	Leaf infusion	[17]; This study
Areca catechu	Seed ingestion	[46, p. 138]
Argemone mexicana	Seed decoction	[47, p. 154]
Artemisia dracunculus	Branch decoction	[48]
Artemisia mexicana		[45, p. 121]
Artemisia vulgaris		[37, p. 68]
Arundo donax	Root-stock decoction	[42]
Asaparagus officinalis	Root-stock decoction	[23, p. 400]
Asaparagus racemosus		[32]
Asarum canadense	Root-stock decoction	[23, p. 400]
Asarum europaeum	Plant decoction	[36, p. 183]

Continued

TABLE 1 Medicinal Plants Used as Diuretics—Cont'd

Species	Preparation	Reference
Asarum sieboldii	Root-stock decoction	[23, p. 400]
Asclepias curassavica	Root decoction	[43, p. 23]
Asplenuim scolopendrium	Plant decoction	[23, p. 400]
Aynodon dactylon	Leaf decoction	[41]
Azardiracta indica	Ground leaf tablets	[46, p. 101]
Bacopa monnieri	Plant decoction	[34, p. 515]
Baliospermum montanum		[32]
Bambusa arundiacea	Root/plant decoctions	[23, p. 401]
Barosma betulina	Leaf decoction	[36, p. 166]
Barosma serratifolia	Leaf extract	This study
Batis maritima	Leaf decoction	[41]
Bauhinia racemosa	Root infusion	[32]
Begonia cucullata	Plant decoction	[34, p. 515]
Begonia sanguinea	Plant decoction	[34, p. 515]
Benincasa hispida	Fruit decoction	[23, p. 401]
Betula pendula	Leaf decoction	[36, p. 164]
Bidens pilosa	Plant decoction	[33, p. 165]
Bidens tripartita	Plant decoction	[23, p. 401]
Bixa orellana	Leaf decoction; powdered leaf tablet	[33, p. 88]
Blumea lacera	Plant decoction	[34, p. 515]
Boerhavia diffusa	Root decoction	[32]
Boerhavia hirsuta	Leaf powder ingestion	[33, p. 118]
Borago officinalis	Leaf infusion	[38]
Borreria verticillata	Plant decoction	[43, p. 121]
Boswellia serrata		[32]
Brassica oleracea	Leaf infusion	[17]
Bryophyllum pinnatum	Leaf decoction	[42]
Buddleia americana	Root/bark/leaf decoctions	[40]

TABLE 1 Medicinal Plants Used as Diuretics—Cont'd

Species	Preparation	Reference
Buddleia marrubiifolia	Plant decoction	[34, p. 515]
Bursera gumifera	Bark and leaf decoction	[41]
Bursera simaruba	Bark decoction	[49, p. 174]
Butea monosperma		[32]
Caesalpinia bonduc	Seed decoction	[48, p. 155]
Calliandria anomala		[45, p. 122]
Callitris arborea	Gum decoction	[34, p. 515]
Calystegia soldanella	Plant decoction	[34, p. 515]
Camellia sinensis	Leaf infusion	[17]; This study
Canna edulis		[41]
Canna indica		[41]
Canna latifolia		[41]
Capriola dactylon	Root-stock decoction	[40]
Capsella bursa pastoris	Aerial parts decoction	[38]
Capsicum annuum	Fruit decoction	[17]
Carex arenaria	Root-stock decoction	[23, p. 402]
Carex hirta	Plant decoction	[34, p. 515]
Carlina acaulis	Root decoction	[36, p. 166]
Cassia fistula	Plant infusion	[33, p. 101]
Cassia occidentalis		[32]
Cassia tora	Leaf decoction	[43, p. 148]
Caulophyllum thalictroides	Root decoction	[23, p. 403]
Cecropia peltata		[49, p. 174]
Cedrus deodara	Bark/wood/oil decoctions	[23, p. 403]
Cedrus libani	Bark/wood/oil decoctions	[23, p. 403]
Centaurea cyanus	Flower decoction	[23, p. 403]
Centella asiatica	Leaf/stem decoctions	[48, p. 67]
Cephalanthus occidentalis	Leaf/stem/flower infusions	This study

Continued

TABLE 1 Medicinal Plants Used as Diuretics—Cont'd

Species	Preparation	Reference
Cestrum nocturnum	Plant decoction	[46, p. 75]
Chamaelirium luteum	Root decoction	[23, p. 404]
Chamaemelum nobile		[37, p. 34]
Chimaphila umbellata	Plant decoction	[23, p. 404]
Chiranthoden pentadactylon		[45, p. 94]
Chloris distichophylla	Fruit decoction	[41]
Chondrodendron tomentosum	Root decoction	[23, p. 404]
Cichorium intybus	Root decoction	[23, p. 404]
Cimicifuga racemosa	Rhizome/root decoctions	[38]
Cissampelos capaeba	Plant decoction	[40]
Cissampelos owariensis	Root decoction	[43, p. 60]
Cissampelos pariera		[32]
Citrullus lanatus	Raw plant consumption	[39, p. 267]
Citrus aurantium	Fruit-juice/rind decoctions	[43, p. 47]
Citrus decumana	Fruit-juice/rind decoctions	[43, p. 47]
Citrus limonum	Fruit-juice/rind decoctions	[43, p. 47]
Citrus sinensis	Fruit juice ingestion	[17]
Claytonia sibirica	Plant decoction	[34, p. 515]
Clematis biondiana	Plant decoction	[34, p. 515]
Clematis gouriana	Plant decoction	[34, p. 515]
Clematis vitalba	Fruit/shoot ingestion	[50, p. 163]
Cocculus pendulus	Root decoction	[43, p. 98]
Cochlearia armoracia	Root decoction	[39, p. 267]
Cochlearia officinalis	Plant decoction	[23, p. 405]
Coffea arabica	Seed decoction	[36, p. 168]
Cola acuminata	Bean decoction/infusion	[12, p. 507]
Collinsonia canadensis	Root/leaf decoctions	[23, p. 406]

TABLE 1 Medicinal Plants Used as Diuretics—Cont'd

Species	Preparation	Reference
Combretum micranthum	Leaf infusion	[43, p. 132]
Consolida regalis	Flower decoction	[23, p. 406]
Convallaria majalis	Leaf/blossom/plant decoctions	[36, p. 176]
Conyza canadensis	Plant decoction	[23, p. 406]
Conyza serpentina		[40]
Copaifera langsdorffii	Wood/oil decoctions	[23, p. 40]
Corchorus olitorius	Leaf decoction	[43, p. 25]
Cordia salicifolia	Powdered leaf infusion	[33, p. 108]
Costus spicatus	Sap decoction	[34, p. 515]
Crataegus mexicana	Root decoction	[40]
Crataegus oxyacantha		[34, p. 359]
Crateva religiosa	Stem–bark decoction	[43, p. 121]
Crescentia linearifolia	Syrup/juice ingestion	[42]
Crithmum maritimum	Plant decoction	[23, p. 406]
Cucumis sativus	Raw plant consumption	[39, p. 267]
Cucurbita maxima	Seed decoction	[43, p. 170]
Cucurbita pepo	Seed decoction	[36, p. 179]
Cynodon dactylon	Root decoction	[46, p. 55]
Cynanchum vincetoxicum	Plant decoction	[34, p. 515]
Cyperus rotundus	Tuber decoction	[43, p. 300]
Cytisus scoparius	Blossom decoction	[36, p. 166]
Daucus carota	Plant/root/fruit decoctions	[17]; This study
Delphinium staphisagria	Flower decoction	[23, p. 407]
Dendrocalamus strictus		[32]
Desmodium gangeticum	Stem and leaf decoction	[43, p. 102]
Dianthus superbus	Plant decoction	[23, p. 408]
Dictamnus albus	Root decoction	[23, p. 408]
Digitalis purpurea	Leaf decoction	[36, p. 171]
Dioscorea cayenensis	Tuber decoction	[45, p. 140]

Continued

TABLE 1 Medicinal Plants Used as Diuretics—Cont'd

Species	Preparation	Reference
Dioscorea villosa	Root-stock/root decoctions	[36, p. 184]
Dipsacus fullonum	Root decoction	[23, p. 408]
Dorstenia brasiliensis		[41]
Drimia indica	Bulb decoction	[34, p. 515]
Elymus repens	Root-stock decoction	[13, p. 233]
Embelia ribes	Leaf decoction	[23, p. 409]
Equisetum arvense	Plant decoction	[11, p. 134]; This study
Equisetum bogotense	Powdered leaf decoction	[40]
Equisetum robustum	Plant decoction	[34, p. 515]
Eriodictyon californicum	Leaf decoction	[23, p. 409]
Eryngium campestre	Plant/root decoctions	[23, p. 409]
Eryngium comosum		[41]
Eryngium maritimum	Plant/root decoctions	[23, p. 409]
Eryngium planum	Plant/root decoctions	[23, p. 409]
Eryngium yuccifolium	Plant decoction	[34, p. 515]
Erysimum cheiri	Flower decoction	[23, p. 409]
Erythrophleum guineense	Bark decoction	[43, p. 28]
Erythrophleum sauveolens	Bark decoction	[43, p. 29]
Eupatorium cannabinum	Plant decoction	[36, p. 173]
Eupatorium perfoliatum	Leaf and flower decoction	[44, p. 322]
Eupatorium purpureum	Plant decoction	[23, p. 410]
Euphorbia hyssopifolia	Plant decoction; sap and leaf infusion	[51]
Euphorbia cyparissias	Plant decoction	[23, p. 410]
Eysenhardtia polystachya	Stem infusion	[41]
Filipendula ulmaria	Blossom/plant decoctions	[36, p. 179]
Foeniculum vulgare	Root /seed decoctions	[38]
Fragaria vesca	Leaf decoction	[36, p. 182]
Fraxinus excelsior	Bark decoction	[36, p. 163]

TABLE 1 Medicinal Plants Used as Diuretics—Cont'd

Species	Preparation	Reference
Fumaria officinalis	Plant decoction	[36, p. 171]
Funtumia africana	Leaf decoction	[43, p. 193]
Gaillardia pinnatifida	Plant decoction	[34, p. 515]
Galega officinalis	Plant decoction	[23, p. 410]
Galium aparine	Plant decoction	[23, p. 411]
Galium verum	Plant decoction	[36, p. 164]
Gaultheria procumbens	Leaf decoction	[36, p. 184]
Genista tinctoria	Plant decoction	[23, p. 411]
Glechoma hederacea	Plant decoction	[23, p. 411]
Globularia alypum	Leaf decoction	[23, p. 411]
Glycine max	Seed decoction	[17]
Gonoplebium incanum		[41]
Gratiola officinalis	Plant decoction	[36, p. 173]
Guaiacum officinale	Wood decoction	[36, p. 175]
Helianthus annuus	Seed decoction	[45, p. 123]
Helichrysum arenarium	Flower decoction	[23, p. 168]
Hemidesmus indicus	Plant decoction	[34, p. 515]
Herniaria glabra	Plant decoction	[36, p. 180]
Herniaria hirsuta	Plant decoction	[23, p. 412]
Hibiscus sabdarifa	Calyx infusion	[41]
Humulus lupulus	Fruit infusion	[17]
Hydrangea arborescens	Root decoction	[13, p. 233]
Hygrophila auriculata	Root/seed decoctions	[34, p. 515]
Hygrophila spinosa	Plant/root decoctions	[23, p. 13]
Hypericum perforatum	Leaf/flower/bud decoctions	[44, p. 338]
Hypochaeris scarzonerae	Plant decoction	[34, p. 515]
Ilex paraguariensis	Leaf decoction	[36, p. 178]
Inula helenium	Root-stock/tuber decoction	[36, p. 169]
Ipomoea turpethum		[32]

Continued

TABLE 1 Medicinal Plants Used as Diuretics—Cont'd

Species	Preparation	Reference
Iresina calea		[41]
Iris germanica		[37, p. 60]
Iris versicolor		[12, p. 507]
Jacaranda procera	Leaf decoction	[23, p. 413]
Jatropha curcas		[49, p. 174]
Jatropha urens	Root decoction	[41]
Juniperus communis	Fruit/berry powder decoctions	[4]
Lamium album	Plant decoction	[36, p. 183]
Laportea meyeniana	Leaf/root decoctions	[34, p. 515]
Larix decidua	Bark/resin decoctions	[23, p. 414]
Laurus nobilis	Fruit/berry decoctions	[36, p. 163]
Lavendula anguistifolia	Dried flower infusion	[44, p. 331]
Leonotis nepetaefolia	Plant decoction	[19]
Lepidium bipinnatifidum	Plant infusion	[41]
Lepidium sativum	Plant/root decoctions	[23, p. 414]
Lepidium virginicum	Plant/root decoctions	[23, p. 414]
Leptadenia hastada	Leaf decoction	[43]
Lespedeza capitata	Plant decoction	[23, p. 415]
Levisticum officinale	Root-stock/tuber/root decoctions	[36, p. 176]
Linaria vulgaris	Plant decoction	[23, p. 415]
Lobelia chinensis	Plant decoction	[23, p. 415]
Lomicera caprifolium	Flower decoction	[23, p. 415]
Luffa aegyptiaca	Fruit decoction	[23, p. 416]
Luffa operculata	Fruit decoction	[23, p. 416]
Lupinus albus	Seed decoction	[23, p. 416]
Lupinus angustifolius	Seed decoction	[23, p. 416]
Lupinus mutabilis	Seed decoction	[23, p. 416]
Lycium halimifolium	Plant decoction	[34, p. 515]

TABLE 1 Medicinal Plants Used as Diuretics—Cont'd

Species	Preparation	Reference
Lycopersicon esculentum	Fruit ingestion	[17]
Lycopodium clavatum	Powdered spore decoction	[23, p. 416]
Malus domestica	Fruit ingestion	[34]
Manilkara zapota		[49, p. 174]
Margirocarpus setosus		[41, p. 141]
Marrubium vulgare		[37, p. 56]
Melia azedarach	Leaf juice ingestion	[43, p. 180]
Melilotus officinalis		[37, p. 38]
Mentha spicata		[37, p. 92]
Mentzelia hispida		[40]
Mercurialis annua	Plant decoction	[23, p. 417]
Mercurialis perennis	Plant decoction	[23, p. 417]
Momordica charantia		[49, p. 160]
Monarda didyma	Plant/blossom decoctions	[36, p. 178]
Morinda geminata	Leaf decoction	[43, p. 63]
Morinda longiflora	Leaf decoction	[43, p. 63]
Morinda lucida	Root–bark decoction	[43, p. 63]
Mucuna pruriens		[32]
Myrrhis odorata	Plant decoction	[23, p. 418]
Nasturtium officinale	Plant decoction	[4]
Nauclea latifolia	Bark decoction	[43, p. 76]
Nerium oleander	Leaf decoction	[43, p. 17]
Neurolaena lobata	Leaf decoction	[51]
Nigella sativa	Seed decoction	[23, p. 418]
Nymphaea lotus	Stem and root infusion	[43, p. 87]
Ocimum basilicum	Plant/leaf decoctions	[17]
Olea europaea	Leaf decoction	[23, p. 419]
Ononis spinosa	Plant decoction	[36, p. 179]
Opilia celtidifolia	Leaf decoction	[43, p. 171]

Continued

TABLE 1 Medicinal Plants Used as Diuretics—Cont'd

Species	Preparation	Reference
Origanum vulgare	Plant decoction	[17]
Orthosiphon aristatus	Plant infusion	[23, p. 419]
Orthosiphon spicatus	Leaf decoction	[36, p. 175]
Orthosiphon stamineus	Leaf decoction	[52, p. 178]
Panax quinquefolius	Plant decoction	[17]
Parietaria judaica	Plant decoction	[23, p. 419]
Parietaria officinalis	Plant decoction	[23, p. 419]
Parquetina nigrescens	Plant decoction	[43, p. 21]
Passiflora jorullensis		[45, p. 121]
Pastinaca sativa	Fruit/root decoctions	[23, p. 420]
Paullinia cupana	Seed decoction	[23, p. 420]
Persea americana		[49, p. 174]
Petasites hybridus	Root-stock/leaf/tuber decoctions	[36, p. 166]
Petroselinum crispum	Seed/root decoctions	[36, p. 178]; This study
Peucedanum ostruthium	Root-stock decoction	[23, p. 420]
Peumus boldus	Leaf decoction	[23, p. 236]
Phaseolus vulgaris	Fruit/berry decoctions	[36, p. 163]
Phyllanthus amarus		[49, p. 174]
Phyllanthus salviaefolius		[41]
Physalis alkekengi	Fruit decoction	[23, p. 420]
Physalis peruviana	Fruit ingestion	[40]
Picria fel-terrae	Leaf decoction	[34, p. 515]
Pimpinella anisum	Seed and oil decoction	[44, p. 321]
Pinus sylvestris	Plant/fruit/berry decoctions	[36, p. 180]
Piper cubeba	Fruit decoction/ powdered leaf infusion	[48]; This study
Piper guineense	Berry decoction	[43, p. 91]
Piper longum	Leaf decoction	[23, p. 421]

TABLE 1 Medicinal Plants Used as Diuretics—Cont'd

Species	Preparation	Reference
Piper methysticum	Root-stock decoction	[53]
Piper umbellatum		[49, p. 174]
Plumeria rubra		[45, p. 123]
Polygonum acre		[41]
Polygonum hydropiper	Plant decoction	[36, p. 181]
Pometia pinnata	Bark decoction	[53]
Populus nigra	Plant/bark/seed decoctions	[36, p. 163]
Populus tremuloides	Bark decoction	[34, p. 515]
Portulaca oleracea	Plant decoction	[23, p. 423]
Pothomorphe peltata	Dry leaves smoked	[42]
Primula veris	Root/blossom decoctions	[36, p. 168]
Prunus avium	Fruit stalk decoction	[23, p. 423]
Prunus cerasus	Fruit juice ingestion	[17]
Prunus spinosa	Blossom decoction	[36, p. 165]
Pseudognaphaliu obtusifolium	Plant decoction	[23, p. 423]
Psoralea corylifolia	Seed decoction	[34, p. 515]
Pulmonaria officinalis	Plant/leaf decoctions	[36, p. 165]
Pyrus communis	Leaf/fruit decoctions	[21, p. 18]
Quercus calliprinos	Bark/acorn decoctions	[11, p. 145]
Rafnia perfoliata	Leaf decoction	[34, p. 515]
Rehmannia lutea	Plant decoction	[34, p. 515]
Rhacoma uragoga		[40]
Rhoeo spathacea	Leaf infusion	[19]
Rhododendron aureum	Leaf decoction	[23, p. 424]
Rhododendron campylocarpum	Leaf decoction	[23, p. 424]
Rhododendron ferrugineum	Leaf decoction	[23, p. 424]

Continued

TABLE 1 Medicinal Plants Used as Diuretics—Cont'd

Species	Preparation	Reference
Rhododendron ponticum	Leaf decoction	[23, p. 424]
Ribes nigrum	Fruit/leaf decoctions	[17]
Rosa canina	Fruit/berry/rind decoctions	[36, p. 169]
Rosmarinus officinalis	Plant decoction	[17]
Rubia tinctorum	Root decoction	[23, p. 425]
Rumex acetosa	Plant decoction	[23, p. 425]
Rumex acetosella	Plant decoction	[23, p. 425]
Ruscus aculeatus	Root-stock/root decoctions	[23, p. 425]
Salvadora persica	Root decoction	[43, p. 209]
Sambucus canadensis	Flower/berry/bark decoctions	[44, p. 326]
Sambucus ebulus	Root-stock/tuber/fruit/berry decoctions	[36, p. 169]
Sambucus nigra	Fruit/berry/bark decoctions	[11, p. 129]
Sambucus simpsonii		[49, p. 160]
Santalum album	Oil decoction	[34, p. 515]
Sassafras albidum	Root-stock/tuber/root decoctions	[36, p. 180]
Scrophularia nodosa	Plant decoction	[23, p. 427]
Scutellaria lateriflora	Aerial part decoction	[38]
Selaginella lepidophylla		[41]
Selaginella pilifera		[41]
Selaginella pringlei		[41]
Selenicereus grandiflorus	Plant decoction	[36, p. 177]
Senecio aureus	Plant decoction	[38]
Serenoa repens	Fruit/berry decoctions	[36, p. 180]
Sinapis alba		[37, p. 70]
Smilax officinalis	Powdered root decoction	[33, p. 175]
Smilax utilis	Root decoction	[36, p. 180]
Solanum dulcamara	Stem decoction	[36, p. 170]

TABLE 1 Medicinal Plants Used as Diuretics—Cont'd

Species	Preparation	Reference
Solanum nigrum		[32]
Solanum seaforthianum		[41]
Solanum surattense	Root decoction	[34, p. 515]
Solanum toryum		[49, p. 174]
Solidago canadensis	Powdered leaf infusion	[11, p. 133]; This study
Solidago virgaurea	Plant decoction	[23, p. 428]; This study
Sopanaria officinalis	Root-stock/root/plant decoctions	[36, p. 182]
Spiranthes diuretica	Plant decoction	[34, p. 515]
Strophanthus kombe	Seed decoction	[36, p. 182]
Tabebuia heterophylla	Leaf ingestion/leaf infusion	[19]
Tagetes erecta		[45, p. 124]
Tagetes lucida		[45, p. 122]
Tagetes multifida	Plant decoction	[34, p. 515]
Taraxacum officinale	Rhizome/root/leaf decoctions	[11, p. 128]
Tephrosia purpurea	Root/leaf/seed decoctions	[43, p. 51]
Terminalia belerica		[32]
Terminalia ivorensis	Powdered bark decoction; bark infusion	[43, p. 203]
Terminalia laxiflora	Bark/leaf decoctions	[43, p. 52]
Terminalia macroptera	Bark/leaf decoctions	[43, p. 52]
Theobroma cacao	Bean decoction	[34, p. 514]
Thespesia populnea		[42]
Thuja occidentalis	Plant decoction	[23, p. 429]
Thymus vulgaris	Plant decoction	[17]
Tilia platyphyllos		[12, p. 507]
Trianthema portulacastrum	Leaf decoction	[34, p. 515]
Tribulus terrestris	Fruit decoction	[34, p. 515]

Continued

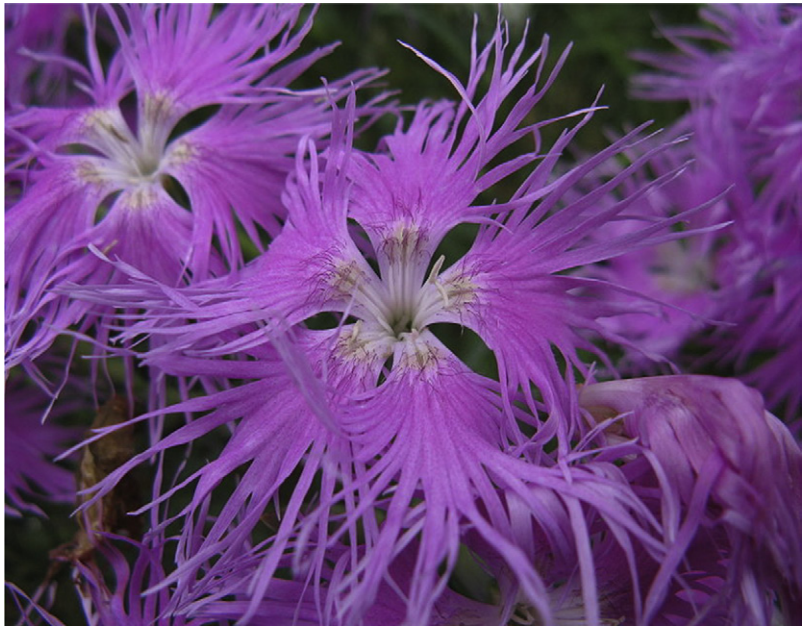
TABLE 1 Medicinal Plants Used as Diuretics—Cont'd

Species	Preparation	Reference
Trigonella foenum-graecum	Seed/seed oil decoctions	[48]
Tropaeolum majus	Leaf decoction	[23, p. 430]
Turnera aphrodisiaca	Leaf infusion	[41]
Turnera diffusa	Leaf decoction	[36, p. 169]
Urginea indica	Bulb decoction	[43, p. 26]
Urginea maritima	Root-stock/tuber d ecoctions	[36, p. 181]
Urtica dioica	Root/plant decoctions	[38]
Urtica urens	Plant tincture/capsule form	[52, p. 176]
Usnea hirta	Whole lichen tincture	[52, p. 177]
Vaccinium macrocarpon	Concentrated juice	[13, p. 231]; This study
Vaccinium myrtillus	Fruit/berry decoctions	[36, p. 164]
Verbascum densiflorum	Flower decoction	[23, p. 431]
Verbascum phlomoides	Flower decoction	[23, p. 431]
Verbena crinoydes		[40]
Verbena officinalis	Plant decoction	[23, p. 431]
Vincetoxicum hirundinaria	Root/root-stock decoctions	[23, p. 431]
Viola tricolor	Plant decoction	[36, p. 178]
Vitis vinifera	Fruit/berry decoctions	[36, p. 172]
Voacanga africana	Leaf decoction	[43, p. 29]
Withania somnifera	Juice ingestion	[43, p. 105]
Zea mays	Flower/pollen decoctions	[13, p. 233]; This study
Zingiber officinale	Powdered root/rhizome infusions	[44, p. 329]

Abutilon indicum
(Photo courtesy of Michael Kesl)

Bixa orellana
(Photo courtesy of Gernot Katzer)

Clematis vitalba
(Photo courtesy of Andreas Bock and Mechthild Scheffer)



Dianthus superbus
(Photo courtesy of I. Kenpei)

Eryngium planum
(Photo courtesy of Roger Holloway of Cottage Gardens, Devon, England)

Erysimum cheiri
(Photo courtesy of Jina Lee)

Galium verum
(Photo courtesy of Margaret Young and The Scottish Rock Garden Club)

Hydrangea arborescens
(Photo courtesy of I. Kenpei)

Inula helenium
(Photo courtesy of Enrico Blasutto)

Monarda didyma
(Photo courtesy of Sten Porse)

Rhoeo spathacea
(Photo courtesy of Forest and Kim Starr)

Tropaeolum majus
(Photo courtesy of Ken McCown)

Several of the diuretic species encountered in the Miami *botanicas* were discovered to serve additional purposes besides inducing diuresis. Three species were portrayed by the proprietors as both diuretics and antilithics (preventive of stone formation): *Aerva lanata, Vaccinium macrocarpon*, and *Equistetum arvense. Turnera aphrodisiaca* acquired its name from its long history of use as a sexual stimulant and aphrodisiac. Besides its role as a source of diuretic phytochemicals, *Clematis vitalba* has been employed since 1930 as one of the 38 botanical species in the homoeopathic medications of the Bach® Flower Remedy system [14]. A highly diluted *Clematis vitalba* preparation is one of the components of the Bach® original Rescue® Remedy "Crisis Formula", and it has been described as helpful in reducing light-headedness and the possibility of fainting during emergencies. It is also recommended to increase focus, attention and concentration in times of severe emotional stress [15,16]. *Clematis vitalba* has been used in folk remedies for cancers, nephrosis, renosis, blood disorders, and ulcers. It has also been reported to be a poison, purgative, rubefacient, sudorific, and vesicant [17]. In these cases and others involving dual-purpose phytomedications, plant parts are selected and preparation methods vary according to the different target functions. Another example is *Spiranthes diuretica*, so named because of the longstanding recognition of its effectiveness in increasing urine excretion. It has also been extensively prescribed in different cultures for blood pressure reduction. This underscores the general observation that many of the plant species in Table 1 that are prescribed for diuresis also serve as a source of ingredients that are applicable for the treatment of other urinary tract conditions and hypertension, for example *Apium graveolens*, *Cucumis sativus, Petroselinum crispum* and *Taraxcum officinale* [18, pp. 154, 217, 224, 481].

Multipurpose diuretic plants ($N = 105$) are listed in Table 2. The most common secondary application is blood pressure regulation. Various phytochemical products in these species act to dynamically modify and balance blood pressure. If hypertension occurs because of blood volume overload, fluid loss would help to reverse it. In several cases the same plant species can serve to either raise or lower blood pressure, depending upon the preparation methods and the anatomical parts selected for processing. These species are classified as blood pressure "regulators", "stabilizers" or "equalizers" by the proprietors of the Miami *botanicas*, and they account for 11% of the species in Table 2. A quarter of the species in Table 2 also have applications in traditional botanical approaches in dentistry. *Argemone mexicana* is another highly versatile diuretic species, with at least 36 other documented medicinal uses ranging from treating ulcers to serving as a strength-building tonic [19]. The most multifaceted species in Table 2 is *Achillea millefollium*. It is prescribed by the herbalists as a diuretic (whole plant decoction), for high blood pressure or hypertension (leaf decoction), and for low blood pressure or hypotension (leaf infusion). According to Moerman *et al*. [20], the latter species has been credited with 359 different therapeutic applications in native North American populations, and it is also exploited for curing in Korea, Kashmir and Mexico. It is ingested to stimulate

TABLE 2 Diuertic Plants Used for High Blood Pressure (HBP), Low Blood Pressure (LBP), Dentistry and Other Medicinal Applications [4,18,19,33,54–57]

Species	Other medicinal applications
Acalypha arvensis	Kidney disease, diarrhoea, ulcers, dermatologic disorders
Achillea millefolium	LBP, HBP, astringent, analgesic, anti-allergenic
Achyranthes aspera	HBP, dentistry
Adonis vernalis	LBP, HBP
Aerva lanata	Antilithic
Aesculus hippocastanum	LBP, HBP, anti-inflammatory, varicose veins
Allium cepa	HBP, dentistry
Allium sativum	HBP, dentistry
Anthriscus cerefolium	HBP
Apium graveolens	HBP
Apocynum cannabinum	HBP
Argemone mexicana	HBP, hepatitis, cancer, syphilis, sedative, contraceptive, emmenagogue
Artemisia dracunculus	Dentistry
Arundo donax	LBP
Azardiracta indica	Dentistry
Bidens pilosa	Analgesic, anti-helminthic, dermatitis, wound repair
Bixa orellana	Diabetes, astringent, poison antidote, antihelmintic
Boerhaavia hirsuta	Asthma, gall bladder disease, anti-inflammatory
Brassica oleracea	HBP
Bryophyllum pinnatum	HBP, asthma, gonorrhoea, ulcers, expectorant, anti-bacterial
Bursera simaruba	LBP, HBP, rheumatism, gout, arthritis, analgesic
Camellia sinensis	LBP, HBP, cancer, anti-oxidant, anti-atherosclerotic
Capsella bursa pastoris	LBP, HBP

TABLE 2 Diuretic Plants Used for High Blood Pressure (HBP), Low Blood Pressure (LBP), Dentistry and Other Medicinal Applications [4,18,19,33,54–57]—Cont'd

Species	Other medicinal applications
Capsicum annuum	HBP
Cassia occidentalis	HBP
Cassia fistula	Laxative, purgative, urinary tract infections, liver disease
Caulophyllum thalictroides	HBP
Cecropia peltata	HBP
Centella asiatica	Dentistry
Cimicifuga racemosa	HBP
Cissampelos pariera	HBP
Citrus aurantium	HBP
Citrus sinensis	LBP, HBP, dentistry
Combretum micranthum	HBP
Convallaria majalis	HBP
Cordia salicifolia	Herpes type 1, appetite stimulant, energy booster
Crataegus oxyacantha	HBP
Cucumis sativus	HBP, urinary tract infections, anti-helminthic
Cynodon dactylon	HBP
Cytisus scoparius	HBP
Daucus carota	HBP
Digitalis purpurea	HBP, antilithic
Equisetum arvense	Dentistry
Equisetum bogotense	Dentistry
Filipendula ulmaria	HBP
Foeniculum vulgare	HBP
Funtumia africana	HBP
Gaultheria procumbens	Dentistry
Genista tinctoria	LBP
Glycine max	HBP

Continued

TABLE 2 Diuretic Plants Used for High Blood Pressure (HBP), Low Blood Pressure (LBP), Dentistry and Other Medicinal Applications [4,18,19,33,54–57]—Cont'd

Species	Other medicinal applications
Guaiacum officnale	HBP, diabetes, arthritis, gout, laxative, analgesic, poison antidote, abortifacient, emmenagogue
Herniaria glabra	HBP
Hibiscus sabdarifa	HBP
Humulus lupulus	LBP, HBP, dentistry
Hypericum perforatum	Anti-depressant, anti-inflammatory, antimicrobial
Lamium album	HBP
Lavendula anguistifolia	LBP
Leonotis nepetaefolia	Malaria, TB, typhoid fever, abortifacient, febrifuge
Lycopersicon esculentum	LBP, HBP
Malus domestica	HBP
Mentha spicata	HBP, gastro-intestinal disease, arthritis, motion sickness
Momordica charantia	HBP, diabetes, rheumatism, abortifacient, febrifuge
Morinda geminata	HBP
Morinda longiflora	HBP
Morinda lucida	HBP
Mucuna pruriens	LBP
Nauclea latifolia	Dentistry
Nerium oleander	HBP
Nigella sativa	HBP, dentistry
Olea europaea	HBP, dentistry
Origanum vulgare	HBP
Orthosiphon spicatus	HBP
Panax quinquefolius	LBP, energy booster
Petroselinum crispum	HBP
Peumus boldus	Choleretic, anti-inflammatory, rheumatism
Phaseolus vulgaris	Dentistry

TABLE 2 Diuertic Plants Used for High Blood Pressure (HBP), Low Blood Pressure (LBP), Dentistry and Other Medicinal Applications [4,18,19,33,54–57]—Cont'd

Species	Other medicinal applications
Pimpinella anisum	LBP, HBP, dentistry
Piper guineense	Dentistry
Piper methysticum	Dentistry, sedative, analgesic, anxiolytic, muscle relaxant
Populus tremuloides	Dentistry
Portulaca oleracea	LBP
Rheo spathacea	Parkinson's disease, rheumatism, gonorrhea, astringent, abortifacient
Ribes nigrum	HBP
Rosmarinus officinalis	LBP, HBP
Salvadora persica	Dentistry
Sambucus ebulus	HBP
Sassafras albidum	Dentistry
Scutellaria lateriflora	HBP, Dentistry
Serenoa repens	Prostate hypertrophy
Smilax officinalis	Kidney disease, syphilis, anti-inflammatory
Solidago canadensis	HBP
Spiranthes diuretica	HBP
Tabebuia heterophylla	Dentistry, cancer, gonorrhea, aphrodisiac, poison antidote
Taraxcum officinale	HBP, urinary tract infections
Tephrosia purpurea	HBP
Terminalia ivorensis	Dentistry
Theobroma cacao	LBP
Thymus vulgaris	HBP
Trigonella foenum-graecum	HBP
Turnera aphrodisiaca	LBP, asthma, aphrodisiac
Urginea maritime	HBP

Continued

TABLE 2 Diuertic Plants Used for High Blood Pressure (HBP), Low Blood Pressure (LBP), Dentistry and Other Medicinal Applications [4,18,19,33,54–57]—Cont'd

Species	Other medicinal applications
Urtica dioica	HBP, arthritis, urinary tract infections, prostate cancer
Vaccinium myrtillus	HBP
Vaccinium macrocarpon	Antilithic
Verbena officinalis	HBP
Vitis vinifera	HBP
Zingiber officinale	HBP, dentistry, anti-oxidant, motion sickness, migraine

appetite, increase biliary secretion, and treat diarrhoea. It is also applied externally as a palliative for liver disorders and as a healing agent for inflammatory skin diseases. The authors note that the genus *Achillea* has yielded over 150 chemical compounds, many of which are useful in medicine.

Several of the Miami *botanicas* stock elemental vitamin and mineral products for diuresis in addition to the botanically based materials. These include the following in tablet, capsule, or powder form: vitamin B-6 (pyridoxine – $C_8H_{11}NO_3$), calcium carbonate ($CaCO_3$), sodium (in the form of sea salt), copper, and zinc. Chlorophyll capsules, derived from organically grown alfalfa, are also recommended. Vitamin B-6, a crystalline phenolic alcohol, is described on a recent commercial website (www.healingfoodreference.com/water_retention.html) as an effective diuretic for individuals experiencing severe water retention. The *botanica* proprietors also recommended moderate intake of coffee (e.g., *Coffea arabica*), other caffeine-containing products, and mild alcoholic beverages to help stimulate diuresis.

Many of the plants in Table 1 contain a sizeable number of bioactive phytochemical compounds that previous research indicates can induce diuresis. According to Duke [17], the following species in Table 1 contain multiple safely ingestible micro-components with diuretic properties: 16 diuresis-inducing chemicals in *Camellia sinensis* leaf infusion tea, 14 in ripe tomatoes (*Lycopersicon esculentum*), 13 in the edible portions of apples (*Malus domestica*), 13 in root and fruit decoctions of carrots (*Daucus carota*), 13 in the juice of sour cherries (*Prunus cerasus*), 13 in the tea produced from a decoction of the entire Rosemary plant (*Rosmarinus officinalis*), 12 in an onion bulb (*Allium cepa*), 12 in bell pepper fruit (*Capsicum annuum*), and 12 in the fruit of the fennel plant (*Foeniculum vulgare*). The same website lists the following nine plants with

11 known phytochemicals with diuretic activity: *Achillea millefolium* (whole plant), *Arctostaphylos uva-ursi* (leaves), *Citrus sinensis* (fruit juice), *Glycine max* (seeds), *Humulus lupulus* (fruit), *Ocimum basilicum* (leaves), *Panax quinquefolius* (whole plant), *Ribes nigrum* (fruit and leaves), and *Thymus vulgaris* (whole plant). *Anethum graveolens* (fruit) and *Brassica oleracea* (leaves) are each characterized by10 diuretic chemicals.

Several of the species compiled in Table 1 contain saponins, complex compounds comprised of a saccharide attached to a steroid or triterpene. The most commonly designated saponin chemical formula is $C_{27}H_{42}O_3$. Extensive research evidence indicates saponins stimulate diuresis [21, p. 77]. In addition, saponins have "hemolytic, expectorative, anti-inflammatory, and immune-stimulating activity" [22]. *Clematis vitalba* is one of the tabulated plants that is rich in saponins. It also contains the glucoside ranunculin ($C_{11}H_{16}O_8$) and the lactone protoanemonin ($C_5H_4O_2$) which are used in traditional Chinese and European medicine to treat skin disorders, arthritis, and rheumatism [23, p. 405]. The wild cucumber (*Cucumis sativus*), originally native to India, has been widely adopted as a diuretic in traditional medical systems around the world. The fruit and seeds contain a substantial quantity of the glycoside cucurbitin ($C_5H_{10}N_2O_2$) which is likely responsible for the plant's diuretic action [18, p. 217].

Another important diuresis-inducing phytochemical is arbutin ($C_{12}H_{16}O_7$). It is found in substantial quantities in the leaves of the pear tree (*Pyrus communis*) and the bearberry shrub (*Arctostaphylos uva-ursi*). Pengelly [21, p. 18] defines arbutin as a phenol, which is a type of aromatic alcohol. It falls within the subclassification of phenylpropanoid phenols. The author states "Arbutin is a urinary tract antiseptic and diuretic…It is indicated for urinary tract infections, in particular cystitis, urethritis, and prostatitis."

The bioactive phytochemicals contained in the plants in Table 1 belong to several chemical classes, the characteristics of which are relevant with respect to their beneficial therapeutic effects. The chemical structures of the bioavailable botanical components found in many of the species described in the present paper can be viewed at the following website provided by the United States Government's National Institute of Health: http://pubchem.ncbi.nlm.nih.gov. As is the case with many previously derived botanical products for other medicinal purposes, opportunities exist for the rapid extraction of diuretic phytochemicals from the species in Table 1 and the formulation of synthetic and semi-synthetic analogues for therapeutic applications.

A number of the diuretic species in Table 1 can potentially produce negative side effects. Frequently observed outcomes are the depletion of potassium and the subsequent unbalanced relationship between electrolytes and body fluids. This is caused by the loss of water, sodium and chloride, especially when diuretic herbs are used chronically or in amounts that exceed recommended dosages [21, p. 18]. Murray *et al*. [24, p. 124] state that "Potassium deficiency can be caused by the use of diuretics, leading to excess fluid loss". The latter authors also note that diuresis-inducing substances can also result in thiamine

(vitamin B-1) deficiency. Thiamine plays a role in the body's energy production in the nervous system by helping to synthesize the neurochemical ester acetylcholine. Thiamine deficiency symptoms include loss of appetite, fatigue, nausea, and mental disturbances such as memory loss and clinical depression [24, p. 104].

A considerable number of species in Table 1 contain potentially poisonous or even fatal phytochemicals. These can be harmful when ingested by themselves or in combination with synthetic or other natural drugs. From a natural selection standpoint, these substances could exist to promote the evolutionary survival of a plant species by discouraging consumption by humans and other animals including insects. Table 3 lists 19 of the diuretic species in Table 1 which have documented toxicity. Parts or extracts of additional diuretic species could be similarly poisonous if ingested in large or undiluted doses. Reported adverse reactions range from skin irritations, to extensive organ and tissue damage, to death. On the other hand, some diuretic plant constituents produce little if any negative side effects. The previously mentioned saponins, for example, do not demonstrate toxic properties in humans [21, p. 77]. On the contrary, saponins have been shown in several controlled studies to decrease blood lipid and glucose levels, inhibit the development of dental caries, reverse acute lead poisoning, and lower the incidence of cancer and renal stones [22,25].

TABLE 3 Diuretic Plants Containing Potentially Toxic Phytochemical Components [28,58–60]

Species	Potential toxicity
Aesculus hippocastanum	Contains aesculin, a toxic courmarin glycoside; nephrotoxicity
Areca catechu	"Betel nut" chewed for stimulant effects; addictive, causes nausea, gingivitis, and can increase risks of cancer and heart disease
Chondrodendron tomentosum	"Curare" contains the alkaloid d-tubocurarine, a muscle relaxant; applied to spear points and arrowheads to immobilize prey; can cause death by respiratory paralysis
Cedrus derodara	Cones produce allergenic pollen
Citrus aurantium	Contains the stimulant synephrine, which can possibly cause fainting, heart rhythm disorders, heart attack, or stroke
Convallaria majalis	Contains cardiac glycosides that can cause severe headaches and cardiovascular disorders including heart failure

TABLE 3 Diuretic Plants Containing Potentially Toxic Phytochemical Components [28,58–60]—Cont'd

Species	Potential toxicity
Cucumis sativus	Can cause electrolyte/fluid imbalance
Cynodon dactylon	Produces what is considered the most allergenic grass pollen
Cytisus scoparius	Contains toxic alkaloids sparteine, isopartine, and hyrdoxytyramine
Erythrophleum guineense	Bark contains various potentially fatal toxic chemicals that can damage the heart and circulatory system
Hypericum perforatum	May significantly reduce antiviral efficacy of blood
Momordica charantia	Seeds and ripe fruit can cause vomiting and purging
Mucuna pruriens	Stinging pod hairs produce severe skin rashes and eye damage
Nerium oleander	Poisonous sap and bark contain oleandin, a potentially fatal cardiac glycoside that can cause nausea, heart damage and prostration
Piper methysticum	Possible liver damage
Serenoa repens	Anti-platelet effect may increase bleeding risk
Solanum dulcamara	Contains toxic glycoalkaloid solanine plus solanidine and dulcamarin
Solanum nigrum	Unripe fruit contains toxic solanine
Urtica dioica	"Stinging nettle" produces urticaria, a painful skin inflammation

On a different level, plant material destined for medicinal use must be stored carefully to prevent spoilage, the growth of toxigenic mold fungi, and other harmful or even poisonous transformations. All potential physiological/medicinal actions of the plant's components and extracts need to be researched and recorded prior to use. Product availability, standardization of herbal potency, dosage variations (e.g., according to age or body weight), limiting conditions, potential interactions with foods and other medications, possible effects of preparation methods, contraindications (e.g., pregnancy), and other influencing factors must be considered.

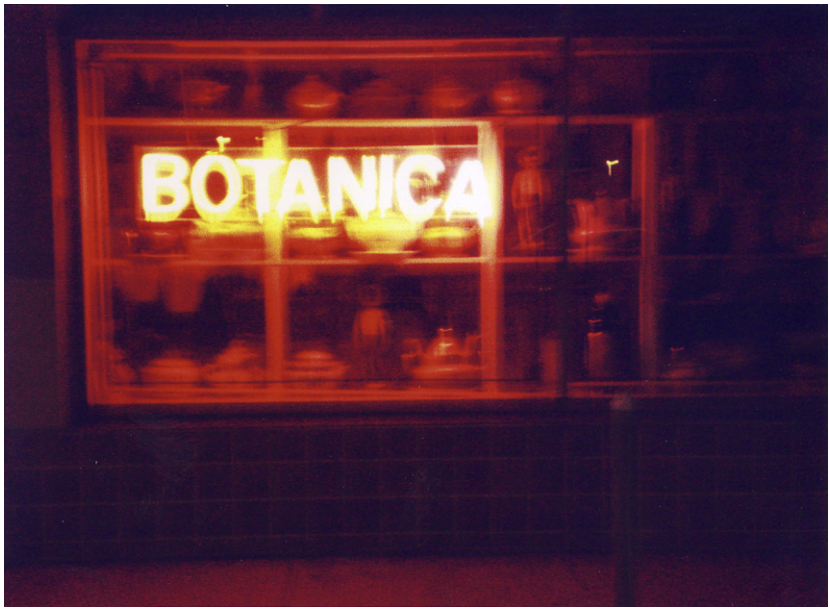


FIGURE 1 Medicinal Plant Shop in Miami.

FIGURE 2 Medicinal Plant Shop with Religious Articles.

FIGURE 3 Medicinal Plant Shop with Life-Size Religious Statues.

FIGURE 4 Medicinal Plant Shop in Miami Offering Health Consultations.

FIGURE 5 Medicinal Plant Shop that Sells Religious Figurines and Images, Bath Products, Books, Candles, Herbs, and Related Containers and Utensils.

DISCUSSION

On a stage set by the ancient history of medicinal plant experimentation, modern field research carries on the discovery of healing botanical products while laboratory investigations continue to verify their biochemical bases. Anthropological evidence indicates that medicinal plant exploitation is a highly adaptive, cross-culturally universal behaviour. Hoffmann [12, p. 10] states "Herbalism is common to all peoples and cultures of the world. The shared experience of alleviating suffering through the use of plant medicines bridges cultural divides, religious differences, and social conflicts." In many populations around the world phytomedicine today remains the "treatment of choice" for a broad spectrum of health problems and serves as the "first line of defense" against potential health hazards in the future. Medicinal plants thus continue to play an indispensable role in both therapeutic and preventive medicine. An increasing number of modern synthetic drugs are botanically based as well. Against this background it has been discovered that in many cultures throughout the world botanical diuretics continue to be recognized as efficacious in treating and preventing excretory system disorders ranging from prostate-related blockages to urinary incontinence.

After years of experimentation in a wide range of societies, it has been revealed that many of the diuretic plants compiled in the present review contain phytochemicals that can deliver positive results. Several alkaloids, flavonoids, saponins, steroids, cardiac glycosides, and phenolics have been shown to induce

diuresis and provide other health benefits. Research indicates that saponin and arbutin not only promote diuresis, they also can act to help eliminate extant infections, strengthen immunity, and reduce inflammation and edema. Botanically derived diuretic substances thus embody the general observation of the multiple therapeutic effects of medicinal plants resulting from the myriad of curative bioavailable chemicals they contain.

As Table 2 illustrates, the complex nature of plant structures and their component chemicals contributes to their documented varied applications. The wide range of phytochemicals found in each species is likely responsible for their multiple documented beneficial outcomes. Briskin [26, p. 508] makes the following statement: "The benefits of phytomedicines often result from synergistic actions of multiple active chemicals." Daniel [27] estimates that there are more than two million bioactive chemical components stored in the plant kingdom. A single plant species can contain several thousand potentially useful biochemical components. The potential medicinal power and healing energy of this natural resource are thus practically unlimited. Plant species contain both primary and secondary metabolites, and generally a low number of the latter are biochemically active. As is the case with all medications, the placebo effect operates to a certain extent to deliver positive outcomes with botanically based treatment regimens.

Time-tested diuretic plant species represent a promising approach to systemic disinfection and curing through the elimination of a wide range of toxins and poisons. By drawing water and contaminated fluids from body cells and increasing urination, diuretics can rapidly flush troublesome disease-producing agents and dietary by-products from a variety of organs and tissues. By altering patterns of urine discharge, they might also be successfully deployed in the treatment of urinary blockages or incontinence. The massive stockpile of diuresis-inducing plant species compiled in the present review attests to the extensive experimentation that has transpired towards achieving this goal. With relatively few side effects in comparison to synthetic drugs, the diluted and distilled botanical preparations represent a relatively safer alternative to prescription diuretics, for example those with the generic names furosemide (trade name = Lasix®; chemical formula = $C_{12}H_{11}CLN_2O_5S$), terazosin hydrochloride (trade name = Hytrin®; chemical formula = $C_{19}H_{25}N_5O_4$.HCL), and tamsulosin hydrochloride (trade name = Flomax®; chemical formula = $C_{20}H_{28}N_2O_5S$.HCL). These drugs are regulated by the U.S. Food and Drug Administration (FDA), while the Dietary Supplement Health and Education Act of 1994 places herbs under the general category of foods rather than drugs and requires these products to be labelled as dietary supplements that can be sold over-the-counter without a prescription [28].

Safety considerations in the use of diuretic herbs will continue to be of paramount importance in the future. Blackwell [29] has documented over 600 plant species that are harmful, toxic, poisonous, or potentially lethal to humans. An updated list containing numerous other toxic or contraindicated species

was recently published [30]. The appropriate use of medicinal plants involves assessing risks and achieving a positive balance between obtaining curative effects and avoiding potential toxicity. Since diuresis-inducing phytochemicals also affect blood pressure, possibly harmful cardiovascular outcomes or other side effects could result from relatively rapid fluctuations in arterial blood flow.

Additional useful plant products, including those prescribed for diuresis, will undoubtedly be discovered in future research. Wang [31] points out that only about 15% of the estimated 300,000 known species of higher plants have been scientifically tested and proven to be therapeutically safe and effective in cross-cultural investigations of medical pharmacopoeias. Rigorous double-blind clinical trials are required to continue the process of verification of the therapeutic value and safety of botanical drugs and medications. Despite the lengthy history of knowledge and use of medicinal plants, in many societies they still remain an underrated and often overlooked important natural resource. Additional chemical and ethnobotanical data are required to identify and verify their purported efficacy.

CONCLUSION

Extensive research and experimentation in different societies has resulted in the discovery of an impressive array of botanical species with chemical components conducive to achieving the beneficial effects of the healthful production and flow of urine. Past trial-and-error by healers in different cultures and recent controlled scientific investigations have paved the way for modern researchers towards fruitful avenues for future discoveries of plant species that can serve as safer sources of diuretics, urinary system modifiers, toxin eliminators, blood pressure stabilizers, and fertility regulators. An important feature of the healers' journey to discovery and verification has been the identification of the appropriate and safe plant parts, preparation methods and combinations, and strategies for maximizing healthful outcomes while reducing the potential for toxicity.

Throughout history and cross-culturally, healers have employed procedures and medicinal substances to expel toxins, poisons, evil spirits, etc. from a sick patient's body. Purging, blood-letting, sweat induction and the use of natural laxatives, expectorants, and diuretics are all prominently featured in traditional medical systems. Diuretic herbs have been frequently prescribed to rid the body of excess fluids through increased urine production, as well as for detoxification and dehydration for such conditions as high blood pressure, kidney infections, obesity, and the edema associated with pre-menstrual syndrome (PMS) or traumatic injuries. A survey of 92 medicinal plant shops ("botanicas") in Miami, Florida discovered 13 botanical species sold specifically for their diuretic effects. An additional 394 species used for this purpose worldwide were catalogued in the literature for a total of 407. These plants contain a number of phytochemical compounds that induce diuresis, including saponin ($C_{27}H_42O_3$) and arbutin ($C_{12}H_{16}O_7$). Research suggests that saponin ingestion is associated with reduced incidence of kidney stones, and arbutin has an anti-bacterial effect on the urinary

tract. Many of the plants contain a number of flavonoids and polyphenols which also may impart therapeutic benefits for the renal system. The two main preparation methods of herbal medicines—decoction and infusion—permit the extraction and concentration of their curative bioactive constituents while simultaneously eliminating or neutralizing toxins, irritants and impurities. The most commonly utilized plant parts to produce traditional diuretic medications are leaves, stems, seeds, roots, berries, barks, saps, resins, pollens, and fruit juices. Many of the plants used as diuretics are also recommended by traditional healers for a variety of other health problems, including high and low blood pressure, dental disorders, and fertility-related applications. Rigorous double-blind/placebo trials are needed to verify the therapeutic value and safety of botanical diuretics.

ACKNOWLEDGEMENTS

Maria Ojeda-Vaz, MPH, MSN, RN of Mt. Sinai Medical Center in Miami, FL assisted in the analysis of data. Melody Pastore, Staff Coordinator of the University of Miami Department of Anthropology, helped with the preparation of the manuscript.

REFERENCES

[1] M.D. Merlin, Econ. Botany 57 (2003) 295–323.
[2] E. Indriati, J. Buikstra, Am. J. Phys. Anthropol. 114 (2001) 242–257.
[3] W.A. Emboden, J. Ethnopharmacol. 3 (1981) 39–77.
[4] R.A. Halberstein, Ann. Epidemiol. 15 (2005) 686–699.
[5] L.L. Barnes, S.S. Sered, Religion and Healing in America. Oxford University Press, New York, 2005.
[6] F.M. Frohock, Healing Powers: Alternative Medicine, Spiritual Communities, and The State. University of Chicago Press, Chicago, IL, 1992.
[7] H.G. Koenig, The Healing Power of Faith. Simon and Schuster, New York, 2001.
[8] N. Gevitz, Other Healers: Unorthodox Medicine in America. Johns Hopkins University Press, Baltimore, 1988.
[9] H.G. Koenig, M. McCullough, D. Larson, Handbook of Religion and Health. Oxford University Press, New York, 2001.
[10] C. Laderman, M. Roseman (Eds.), The Performance of Healing, Routledge, New York, 1996.
[11] E. Torres, Healing with Herbs and Rituals: A Mexican Tradition. University of New Mexico Press, Albuquerque, NM, 2006.
[12] D. Hoffmann, Medical Herbalism: The Science and Practice of Herbal Medicine. Healing Arts Press, Rochester, VT, 2003.
[13] E. Yarnell, K. Abascal, R. Rountree, Clinical Botanical Medicine. Mary Ann Liebert Publishers, New Rochelle, NY, 2009.
[13a] C. Wright, L. Van Buren, C. Kroner, M. Konig , J. Ethnopharmacol. 114 (2007) 1–31.
[14] F. Mantle, Complement. Ther. Nurs. Midwifery 3 (1997) 142–144.
[15] R.A. Halberstein, L. DeSantis, A. Sirkin, V. Padron-Fajardo, M. Ojeda-Vaz, Complement. Health Practice Rev. 12 (2007) 3–14.
[16] Bach Centre (UK); http://www.BachCentre.com (2011).

[17] J.A. Duke, Dr, Duke's phytochemical and ethnobotanical databases. http://www.ars-grin.gov/duke/ (2011).
[18] L. Skidmore-Roth, Mosby's Handbook of Herbs and Natural Supplements, fourth ed., Mosby/Elsevier, St. Louis, 2010.
[19] R.A. Halberstein, J. Altern. Complement. Med. 3 (1997) 227–239.
[20] D. Moerman, R. Pemberton, D. Kiefer, J. Ethnobiol. 19 (1999) 49–67.
[21] A. Pengelly, The Constituents of Medicinal Plants, second ed., CABI Publishers, Cambridge, MA, 2004.
[22] R. Sahelian, Saponins in plants: Benefit and side effects. http://www.raysahelian.com/saponin.html (2011).
[23] B.E. Van Wyk, M. Wink, Medicinal Plants of the World. Timber Press, Portland, OR, 2004.
[24] M. Murray, J. Pizzorno, L. Pizzorno, The Encyclopedia of Healing Foods. Atria Books, New York, 2005.
[25] J. Shi, K. Arunasalam, D. Yeung, Y. Kakuda, G. Mittal, Y. Jiang, J. Med. Food 7 (2004) 67–78.
[26] D.P. Briskin, Plant Physiol. 124 (2000) 507–514.
[27] M. Daniel, Medicinal Plants: Chemistry and Properties. Science Publishers, Enfield, NH, 2006.
[28] M.A. Fitzgerald, Am. Nurse Today 2 (12) (2007) 27–32.
[29] W. Blackwell, Poisonous and Medicinal Plants. Prentice-Hall Publishers, Englewood Cliffs, NJ, 1990.
[30] R. Bevan-Jones, Poisonous Plants: A Cultural and Social History. Windgather Press, Oxford, UK, 2009.
[31] M. Wang, Biological screening of medicinal plants. In: Z. Yaniv, U. Bachrach (Eds.), Handbook of Medicinal Plants, Haworth Press, New York, 2005, pp. 213–233.
[32] N.G. Patel, Ayurveda: the traditional medicine of India. In: R.P. Steiner (Ed.), Folk Medicine: The Art and The Science, American Chemical Society, Washington, DC, 1986, pp. 41–66.
[33] A.M. DeStephano, Latino Folk Medicine: Healing Herbal Remedies from Ancient Traditions. Simon and Schuster, New York, 2001.
[34] W.H. Lewis, M.E.; Lewis, Medical Botany, second ed., Wiley, New York, 2003.
[35] C. Yuan, E. Bieber, Textbook of Complementary and Alternative Medicine. Parthenon (CRC) Press, Boca Raton, FL, 2003.
[36] W. Thomson, Medicines From the Earth: A Guide to Healing Plants. McGraw-Hill, New York, 1978.
[37] M. Heilmeyer, Ancient Herbs. Getty Publications, Los Angeles, 2007.
[38] E.Q. Youngkin, D.S. Israel, Nurs. Pract. 21 (1996) 39–62.
[39] J. Wilen, L. Wilen, Healing Remedies. Ballantine Books, New York, 2008.
[40] H.H. Hirschhorn, J. Ethnopharmacol. 5 (1982) 163–180.
[41] H.H. Hirschhorn, J. Ethnopharmacol. 4 (1981) 129–158.
[42] S.H. Colon, Ethnomedicine 4 (1977) 139–167.
[43] B. Oliver-Bever, Medicinal Plants in Tropical West Africa. Cambridge University Press, Cambridge, UK, 1986.
[44] J. Kowalak, N. Chohan, S. Follin, Nurse's Handbook of Alternative and Complementary Therapies. Lippincott Williams & Wilkins, Philadelphia, 2003.
[45] N.L. Etkin, Plants in Indigenous Medicine and Diet: Biobehavioral Approaches. Redgrave Press, Bedford Hills, NY, 1986.
[46] K. Mahabir, Medicinal and Edible Plants Used By East Indians of Trinidad & Tobago. Chakra Publishers, San Juan, Trinidad & Tobago, 1991.

[47] J. Lindley, Medical and Economic Botany. Bradbury & Evans, London, 1849.
[48] E. Lev, Z. Amar, J. Ethnopharmacol. 72 (2000) 191–205.
[49] A. Payne-Jackson, M. Alleyne, Jamaican Folk Medicine. University of West Indies Press, Kingston, Jamaica, 2004.
[50] D.J. Mabberly, The Plant-Book, A Portable Book of Vascular Plants. Cambridge University Press, Cambridge UK, 1997.
[51] C.G. Coe, J. Ethnobiol. 28 (2008) 29–38.
[52] W.A. Mitchell, Plant Medicine in Practice. Elsevier Science, Philadelphia, 2003.
[53] R.C. Cambie, Fijian medicinal plants. In: R.P. Steiner (Ed.), Folk Medicine: The Art and The Science, American Chemical Society, Washington, DC, 1986, pp. 67–90.
[54] R.A. Halberstein, Cult. Med. Psychiat. 2 (1978) 177–203.
[55] R.A. Halberstein, J. Caribbean Stud. 22 (2008) 87–111.
[56] R.A. Halberstein, Nat. Prod. Commun. 3 (2008) 1813–1824.
[57] K. Magee, C. Loiacono, Int. J. Dent. Hygiene 2 (2004) 111–121.
[58] A.D. Marderosian, L.E. Liberti, Natural Product Medicine. George F. Stickley Co., Philadelphia, 1988.
[59] Natural Medicines Comprehensive Database; http://naturaldatabase.therapeuticresearch.com (2011).
[60] A. Stewart, Wicked Plants. Algonquin Books. Chapel Hill, NC, 2009.

Chapter 2

The *Artemisia* L. Genus: A Review of Bioactive Sesquiterpene Lactones

María José Abad Martínez, Luis Miguel Bedoya Del Olmo, Luis Apaza Ticona and Paulina Bermejo Benito

Department of Pharmacology, Faculty of Pharmacy, University Complutense, Ciudad Universitaria s/n, Madrid, Spain

INTRODUCTION

The use of plants as medicines goes back as far as early man. Certainly the great civilisations of the ancient Chinese, Indians and North Africans provided written evidence of man's ingenuity in utilising plants for the treatment of a wide variety of diseases. Medicinal plants are nature's gift to human beings for making a disease-free healthy life, and play a vital role in preserving our health. It was not until the nineteenth century that man began to isolate the active principles of medicinal plants and one particular milestone was the discovery of quinine from *Cinchona* L. bark by Pelletier and Caventou in 1820. This discovery led to an interest in plants from the New World and expeditions scoured the almost impenetrable jungles and forests in the quest for new medicines. Laboratories became centres for isolating the active principles of medicinal plants from around the globe. Prior to World War II, a series of natural products isolated from higher plants became clinical agents, and a number are still in use. Despite these discoveries, the impact of phytochemistry on new drug development waned and inevitably the innovative pharmaceutical industry turned to synthetic chemicals.

However, during recent years the attention of the pharmaceutical industry has switched once more to the natural world, as illustrated by referring three clinical drugs, taxol, etoposide and artemisinin. Artemisinin, Fig. 1, is an unusual sesquiterpene lactone endoperoxide that has been isolated as the active principle of the Chinese antimalarial herb *Artemisia annua* L. Medicinal herbs from traditional Chinese medicine, such as *Artemisia* genus, hold a unique

Bioactive Natural Products (Part Q), Vol. 37. http://dx.doi.org/10.1016/B978-0-444-59514-0.00002-X

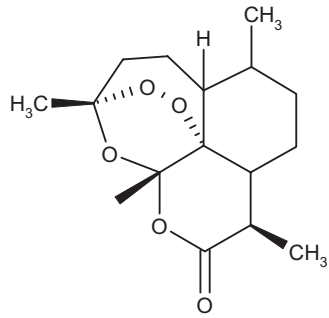


FIGURE 1 Structure of artemisinin.

position since this enormous variety of drugs of plant origin is founded on more than 5000 years of tradition.

The genus *Artemisia* L., usually represented by small herbs and shrubs, is one of the largest genera in the Compositae family consisting of more than 500 species, which is predominantly distributed in the northern temperate region of the world in the 0–50cm precipitation area. Many species have been used since ancient times as folk remedies in various treatments (reducing phlegm, relieving cough, invigorating blood circulation, stopping pain, inducing sweat, diuresis, antihypertension, anthelmintic, antitoxic and antiallergy). According to the literature, over 260 *Artemisia* species have been investigated to reveal that they contain many classes of secondary metabolites, including sesquiterpene lactones. As reported, some substances from the genus were shown to be antimalarial, antiviral, antitumour, antipyretic, antihemorragic, anticoagulant, antianginal, antioxidant, antiulcerogenic and antispasmodic [1].

Different families of sesquiterpene lactones have been reported from this genus, eudesmanolides, guaianolides and germacranolides being the most common. Among the major therapeutical applications of this class of compounds, artemisinin from *A. annua* is undoubtedly a lead compound as a potent antimalarial agent [2–6]. Clinical trials have demonstrated that artemisinin is an effective antimalarial drug and can be used to treat infections of multidrug-resistant strains of *Plasmodium falciparum*, the cause of human malignant cerebral malaria. Since the discovery of artemisinin and its successful clinical trials as an antimalarial drug, there has been an increasing interest in the genus *Artemisia*. This genus has a medicinal history reaching back over two millennia and some of its extracts and isolates are of proven efficacy, officially recommended by the World Health Organization.

This review will focus, after an ethnopharmacological presentation of the genus, on the significant achievements of recent years (2000 to date) regarding the chemical and biological properties of sesquiterpene lactones from the *Artemisia* species, with particular attention to their antimalarial, cytotoxic and anti-inflammatory effects. The discussion will also focus on the understanding of their mechanism of action and structure/activity relationships.

ETHNOPHARMACOLOGICAL USES OF *ARTEMISIA* SPECIES

Many general traditional medicinal uses of *Artemisia* include treatments for wounds and ulcers, headaches, gastrointestinal illnesses and bacterial/fungal infections. Some *Artemisia* species have also been integral components of traditional oriental medicines, and have generally been used as spasmolytics, analgesics and sedatives for symptoms associated with nervous disorders. One of the primary uses of several *Artemisia* species and the traditional medicines derived from them is the treatment of hypertension, diabetes and malaria; more details are given below [7–21].

Preparations of *Artemisia abrotanum* L. ("southernwood") have been used in traditional medicine for treating a variety of disorders and diseases, including upper airway diseases. Some of the earliest Greek and Roman traditional medicinal sources of southernwood include its uses as an anti-inflammatory or spasmolytic, to clear respiratory passages and facilitate breathing. This knowledge also dates back to medieval and Renaissance medicine, and was further developed and refined over centuries of use in European medicine.

Herba *Artemisia absinthium* L., also known as "wormwood", is described in books of pharmacopoeia in many countries around the world, and is certainly of ethnopharmacological interest. It is known by a variety of names depending upon the country. Wormwood enjoys widespread use and was held in high esteem among the ancients. The name "wormwood" is derived from its anthelmintic properties, which were recognized by the ancient Egyptians. Wormwood, with regard to its bitter taste, is mentioned several times in the Bible. The Greek word "apsinthion", meaning "undrinkable", is likely the ancestor of the word "absinthe", which is used in French for the plant species, as well as for the alcoholic beverage. The Greek mathematician and philosopher Pythagoras recommended wine-soaked wormwood leaves to alleviate labour pains; Hippocrates used wormwood extracts for the treatment of menstrual pain and rheumatism.

Artemisia abyssinica Schultz-Bip., known as "chikugn" in Ethiopia, is quite commonly used in traditional medicine and in rituals, especially during the Ethiopian New Year and "Meskel" (the finding of the "true cross") festivities. It is reported as being used as a remedy for heart troubles and as a cough cure. It is also indigenously employed for treatment of rabies, tonsillitis, gonorrhoea, cough, syphilis and leprosy. In Saudi Arabia, the decoction of the fresh whole plant is traditionally used to treat diabetes mellitus.

Artemisia afra (Jacq. Ex. Willd) (known as "koddoo-adi" in Ethiopia) is widely used in combination with other herbs as a remedy against headache, eye disease, haematuria and stabbing pain. It is also employed alone as antifertility agent.

Artemisia annua L. (known as "quinghao" in China) is a common weed over large parts of eastern Europe and Asia, and has become naturalized in North America. It is cultivated on commercial scale in eastern China, in the Balkans, and more recently in India and Africa. In Chinese traditional medicine, it is used

for the treatment of malaria, haemorrhoids and fever. Nowadays, this plant is being cultivated on experimental basis in Ethiopia, and is sold as herbal tea for the treatment of malaria in Ethiopia and in many other countries of Africa.

Artemisia anomala S. Moore (Chinese name "nan-liu-ji-nu") has been used for centuries in Chinese folk medicines for its functions as an analgesic, haemostatic and antibiotic and for curing wounds.

Artemisia apiacea Hance is widely distributed on wasteland and river beaches of Korea, Japan and China. The plant has been used as traditional medicine to treat eczema, jaundice, fever and alopecia.

Artemisia arborescens L. is an aromatic plant endemic in Mediterranean regions and very common in Sardinia, which has been used as a treatment for inflammation, headache and to alleviate urticaria, rheumatic pain, neuralgia and gastrointestinal pain. It is also used as a bronchodilator and as a lenitive of renal colic and haematic fits caused by favism.

Artemisia argyi Levl. et Vant., whose leaves are known as "gaiyou", is a widely used traditional Chinese medicine. The plants are used to treat eczema, inflammation, haemostasis, menstruation-related problems and tuberculosis.

Artemisia asiatica Nakai is widely used as a traditional Asian medicinal plant for the treatment of gastritis; it also has an anti-inflammatory effect.

Artemisia brevifolia Wall. ex DC., locally named as "afsanteen" is widely used in ethno-veterinary medicine system in Pakistan as an anthelmintic.

Artemisia capillaris Thunb. has been used for the treatment of liver diseases such as hepatitis, jaundice and fatty liver in traditional oriental medicine.

Artemisia copa Phil, commonly known as "copa-copa", is a small bush that grows in northwest Argentina and in northern Chile. The plant is regularly sold in local markets and herbal health food stores, and the infusion of the aerial parts are used in popular medicine as an antitussive, digestive, for lowering fever, pulmonary diseases and hypertension.

Artemisia diffusa Krasch. ex Poljak is used by local people in Iran as an anthelmintic. The plant cooked in water is used to promote wound healing. The decoction extract is topically administered to cure infectious wounds.

Artemisia douglasiana Besser ("California mugwort") is a common perennial herb found throughout the western United States, especially northern California, Oregon and Washington. The plant is used to promote menstruation, as a stimulant, tonic, to treat nervous disorders and as a diuretic. In Argentina, *A. douglasiana* leaves are used in folk medicine, where the plant is known by the vernacular name of "matico". The popular use of the infusion is as a cytoprotective agent against peptic ulcer, and it is also used for the treatment of external sores.

Artemisia dracunculus L. or "Russian tarragon" is a perennial herb. Many edible and medicinal uses have been attributed to this species, and it has been commonly used in many recipes for flavouring food for centuries. *A. dracunculus* is a close relative of the French or "cooking tarragon". The plant is known for its anticoagulant and antiepileptic activities in Iranian folk medicine. Due to the mild sedative nature of the plant, it has also been used as a sleep aid.

A. dracunculus has also a traditional Persian history of use as an agent to correct the hyperglucemia associated with the diabetic condition, and for the treatment of headaches and dizziness.

Artemisia feddei LEV. et VAN. is a perennial herb growing in Korea. The aerial parts have been used in folk medicine in oriental countries as an anti-inflammatory, choleretic, antipyretic and diuretic agent, and against digestive disorders.

Artemisia fukudo Makino is distributed along the shorelines of South Korea's Jeju Island and in the south of the Korean Peninsula, in Japan and Taiwan. This plant is used as a flavouring agent and in a variety of cosmetics in Korea. It also has various medicinal uses, including as an anti-inflammatory, antitumour and antibacterial agent.

Artemisia genipi Weber, a mountain wormwood, is used for the production of the celebrated alpine liqueur genepy.

The endemic Cape Verdean *Artemisia gorgonum* Webb is used in local folk medicine as a treatment for symptoms associated with fever.

Artemisia herba-alba Asso. is widely used in Algerian folk medicine for treatment of gastric disorders such as diarrhoea, abdominal cramps and for healing external wounds. Uses for diabetes mellitus and other conditions such as jaundice are also reported, and the species is recommended for neurological disorders.

Artemisia iwayomogi Kitamura is a perennial small herbal plant that has long been used as a chemopreventive agent in traditional Korean medicine. The aerial parts of *A. iwayomogi* have been used in oriental traditional medicine to cure various infectious diseases such as carbuncle, sores, colecistitis and hepatitis, and to treat fever, inflammation and jaundice.

Artemisia keiskeana Miq. grows as a perennial herb in mountainous areas of Korea and is widely distributed. The plant has been used as a traditional crude drug for the treatment of gynaecopathy, amenorrhoea, bruising and rheumatic diseases.

The inhabitants of north-eastern Mexico use an infusion of leaves of *Artemisia ludoviciana* Nutt as an antidiarrhoeal remedy. People also drink the infusion to alleviate the pain caused by hepatic colic in gallstone cases due to its antispasmodic action, as well as being anthelmintic, stomachic, appetizer, regulator of menstruation and antimalarial.

Artemisia minor Jacq. ex Bess. grows uniquely on the Qinghai–Tibet plateau in China. It has long been used for the treatment of fever, rheumatism, dysentery, scabies and bruising.

Artemisia oliveriana J. May ex DC. is used by local people in Iran as an anthelmintic.

Artemisia ordosica Krasch. is widely distributed in dry areas of east Asia. The aerial part is very often utilized in Mongolian folk medicine for the treatment of laryngitis, pharyngitis, haemorrhage and rheumatoid arthritis.

The species *Artemisia princeps* Willd ("dietary wormwood") is a common herb widely spread in northeast Asian countries. The decoction of the herb has been used

as health food and for the treatment of inflammation, diarrhoea and circulatory disorders and as a hepatoprotective and antibacterial agent in Korea, China and Japan.

Artemisia rubripes Nakai has been used as a traditional Korean medicine for stomach-ache, vomiting, diarrhoea and as a haemostatic agent.

Artemisia santonicum L., which is known as "deniz yausani" and "kokulu yausan", grows in sandy places and salted land in Turkey. The plant has been used as an anthelmintic and in the treatment of diabetes.

Artemisia scoparia Waldst. & Kit. ("redstem wormwood") is a faintly scented annual herb widespread and common throughout the world, particularly in southwest Asia and Central Europe. It possesses antibacterial, anticholesterolemic, antipyretic, antiseptic, cholagogue, diuretic, purgative and vasodilator activity, and has also been used for treatment of gall bladder inflammation, hepatitis and jaundice, and as an insecticidal agent.

Artemisia sphaerocephala Krasch is an important resource in north-western China, and has potential pharmaceutical value and an environmental protection function.

Artemisia spicigera C. Koch., locally named "yausan" in Turkish, is widespread in middle and eastern Anatolia, and is traditionally used for skin diseases and ulcerative sores. The dried leaves of this plant are also used to help induce more rapid healing of wounds and for the treatment of eczema and herpes.

Artemisia sylvatica Maxim. has been used in Chinese natural medicine and is prescribed as both a haemostatic and sedative agent in Chinese traditional preparations.

Artemisia thuscula Cav., a synonym of *Artemisia canariensis* (Bess.) Lessing, is an endemic Canary Islands species which has been used in local Canarian folklore medicine as a panacea for a great diversity of health problems. Traditionally this plant has been used as a diuretic, hypoglycaemic, antidiarrhoeic, uricosuric, spasmolytic, stomachic, carminative, vermifuge, tranquilizer, pectoral and anticatarrhal agent, and is still used today. Furthermore, it is used as a mosquito repellent and for other harmful insects or pests of people or harvest crops, by means of aromatic smoke.

In the western United States, *Artemisia tripartita* Rydb. ("three-tip sagebrush") is a native species that has been used by native Americans in the treatment of colds, sore throats, tonsillitis, headaches and wounds.

The bitter vegetable "mountain wormwood", *Artemisia umbelliformis* Lam., is used for the production of genepy, the celebrated bitter alpine liqueur, recently granted a Geographical Indication status by the European Community.

Artemisia vetista Wall is distributed on wasteland and river beaches of China, and has been widely used in traditional Tibetan and Chinese medicine for treating various inflammatory diseases.

Artemisia vulgaris L. is a medicinal herb found throughout the Philippines. The juice of the leaves is used as a vulnerary, while a decoction of the leaves and flowering tops is considered to be an expectorant. The leaves are also considered to be carminative and emmenagogue.

SESQUITERPENE LACTONES FROM *ARTEMISIA* GENUS

Sesquiterpene lactones are a colourless, bitter and stable subfamily of terpenoids, a class of secondary metabolites with a lipophilic character. They are a diverse group of terpenoids with a characteristic isoprenoid ring system, a lactone ring containing a conjugated exomethylene group (α-methylene-γ-lactone). Sesquiterpene lactones constitute a large and diverse group of biologically active plant constituents that are emerging as one of the largest groups of plant products, with over 3000 naturally occurring substances known. They are almost exclusively derived from Asteraceae, but are also found in Umbelliferae and Magnoliaceae. With a few exceptions, the active sesquiterpene lactones contain an exocyclic α,β-unsaturated lactone moiety. The classification of the different skeletal types of lactones is based on the carbocyclic skeletons in which the suffix "olide" indicates the presence of a lactonic function.

Sesquiterpene lactones are 15-carbon compounds consisting of three isoprene units and a lactone group (cyclic ester). They can be categorized, relative to their carbocyclic skeleton, into the following major groups: germacranolides (10-membered ring); eudesmanolides and eremophilanolides (all 6/6-bicyclic compounds); and guaianolides, pseudoguaianolides and hypocretenolides (all 5/7-bicyclic compounds). The germacranolides can be considered the biogenetic precursors for the other skeletal types of lactones, and represent the largest group.

The most widespread sesquiterpene lactones reported in the *Artemisia* genus are guaianolides, but germacranolides- and eudesmanolides-type have also been isolated. Artemisinin, Fig. 1, is an unusual sesquiterpene lactone endoperoxide abundant in *A. annua*, to which the medicinal properties of this herb are attributed [8,11]. This compound has also been isolated from other *Artemisia* species such as *A. scoparia* [22]. Sesquiterpene lactones chemically related to artemisinin have also been identified from other *Artemisia* species. Artemisolide, Fig. 2, is a new class of sesquiterpene lactone with a cyclopropane ring system. This compound, together with arteminolides A, B, C and D, Fig. 3, has been isolated from *A. argyi* [23,24].

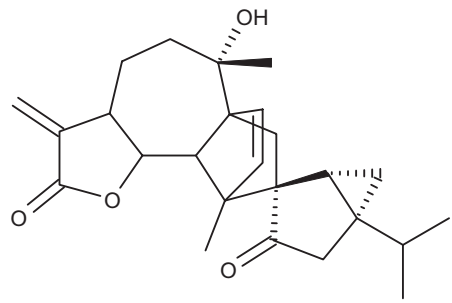


FIGURE 2 Structure of artemisolide.

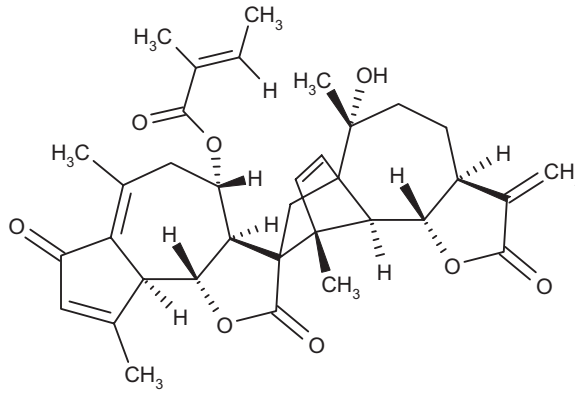


FIGURE 3 Structure of arteminolide D.

However, a survey of currently available chemical data suggests that guaianolic-types are the main classes of sesquiterpene lactones in *Artemisia* species. Some of these compounds were isolated by bioassay-guided fractionation, after previously detecting activity on the part of the plant. Three new guaianolide sesquiterpene lactones, together with six known sesquiterpenes, were isolated by bioassay-guided fractionation from the methanol extract of the aerial parts of *A. sylvatica* [25]. From *A. anomala*, Wen *et al*. [26] isolated a new dimeric guaianolide, Fig. 4, together with several known sesquiterpene lactones. This species also yielded several 1,10-secoguaianolides such as secotanapartholide C, Fig. 5, 3-methoxytanapartholide, 3-*O*-methyl-iso-secotanapartholide and 2α-chloro-iso-secotanapartholide [27].

Investigations into the Chinese folk medicine *Artemisia dubia* Wall. have resulted in the isolation of eight new guaianolides, artemdubolides A–H [28], while *Artemisia suksdorfii* Piper, a native perennial of the coastal Pacific northwest area of the United States, yielded seven new sesquiterpene lactones of the guaianolide structural type [29]. The chromatographic separation of a

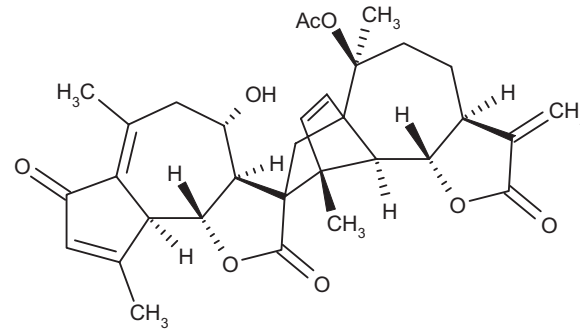


FIGURE 4 Structure of a new dimeric guaianolide from *Artemisia anomala.*

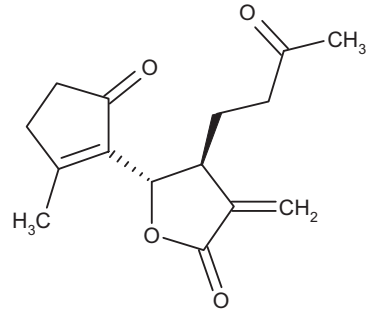


FIGURE 5 Structure of secotanapartholide C.

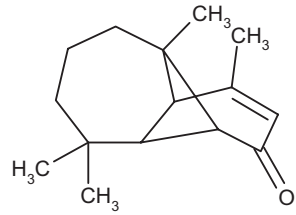


FIGURE 6 Structure of vulgarone B.

methylene chloride extract of *A. rubripes*, a Korean traditional medicine, led to the isolation of a new sesquiterpene lactone, 1α,4β-dihydroxy-8α-acetoxy-guaia-2,10(14),11(13)-triene-6,12-olide [30].

Vulgarone B, Fig. 6, is a sesquiterpene ketone abundant in *A. iwayomogi*, to which the antimicrobial properties of this herb are attributed [31]. This compound has also been isolated as the active constituent of *A. douglasiana* [32]. This species also yielded the antimicrobial sesquiterpene lactone dehydroleucodine [33].

Besides germacranolides and guaianolides, reports on the identification of sesquiterpene lactones of eudesmanolide type in *Artemisia* genus have also been found in the literature, such as 1β,6α-dihydroxy-4(15)-eudesmene isolated from *A. rubripes* [30]. Examples of other sesquiterpene lactones isolated from *Artemisia* genus also included guaianolides, secoguaianolides and germacranolides isolated from *A. gorgonum*, an endemic Cape Verdean *Artemisia* species [34].

PHARMACOLOGICAL ACTIVITY OF *ARTEMISIA* GENUS

Antimalarial and Other Anti-infective Activities

Malaria is an age-old disease which has had a large influence on the economies and development of nations for millennia. Despite major international efforts, malaria still inflicts an enormous toll on human lives, especially in Africa [35].

This disease is a global health problem that threatens 300–500 million people and kills more than one million people annually.

Today we know that malaria is a vector-borne infectious disease caused by the protozoan Plasmodia parasite. There are four types of *Plasmodium* species namely *P. falciparum, Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*, while the vector carrying and transmitting the disease is the female of the *Anopheles* mosquito species. It is widespread in tropical and subtropical regions, including parts of the Americas, Asia and Africa. Malaria is commonly associated with poverty, but it is also a cause of poverty and a major hindrance to economic development. Mortality, currently estimated at over one million people per year, has risen in recent years, mainly due to increasing resistance to antimalarial medicines [36].

Malaria control requires an integrated approach consisting mainly of prevention, including vector control and the use of effective prophylactic antimalarials, and treatment of infected patients with effective antimalarials [37]. Throughout history, antimalarial medicines have been one of the most powerful tools in malaria control. However, the emergence and spread of parasite strains that are resistant to multiple antimalarial drugs has become one of the greatest challenges to malaria treatment, and is associated with the increase in morbidity and mortality in many malaria-endemic countries. The antimalarial chloroquine, formerly a mainstay of malaria control, is now ineffective in most *P. falciparum* malaria areas, and resistance to other antimalarials is increasing rapidly. To deal with this grave situation, artemisinin-based combinatory therapies (ACTS) have been introduced and widely deployed in malarial regions.

The Chinese medicinal plant *A. annua* (“qinghao”) is the known source of the sesquiterpene lactone artemisinin, Fig. 1 (“qinghaosu”), which is used in the treatment of malaria [38]. Artemisinin is a highly oxygenated sesquiterpene lactone, containing a unique 1,2,4-trioxane ring structure, which is responsible for the antimalarial activity of this natural product. Clinical trials have demonstrated that artemisinin is an effective antimalarial and can be used to treat infections of multidrug-resistant strains of *P. falciparum*. Since artemisinin was discovered to be the active component of *A. annua* in the early 1970s, hundreds of papers have focused on the antiparasitic effects of artemisinin and its semi-synthetic analogues dihydroartemisinin, artemether, Fig. 7, arteether and artesunate [39]. Some of the semi-synthetic derivatives, including artemether (the methyl ether of dihydroartemisinin) have improved pharmacokinetic properties and are also of current clinical use. As described above, the active moiety of artemisinin is 1,2,4-trioxane, and a series of synthetic analogues show marked activity against *Plasmodium* species *in vivo* and *in vitro*.

Nowadays, artemisinin and its derivatives have become essential components of antimalarial treatment, and these ACTS are recommended by the World Health Organization to treat especially multidrug-resistant forms of malaria. Drug combinations based on artemisinins offer an effective possibility to counteract drug resistance.

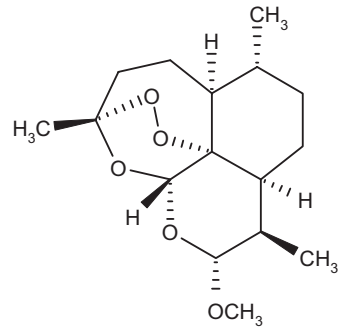


FIGURE 7 Structure of artemether.

There is considerable debate regarding the mechanisms of antimalarial action of artemisinins. *P. falciparum* multiplies in red blood cells, and digestion of haemoglobin during its 48h asexual life cycle is essential for parasite survival. For many years, artemisinins have been proposed to act on parasite haemoglobin-digestion processes within the "food vacuole". Other studies have indicated that artemisinins could also target the parasite mitochondrian or the translationally controlled tumour protein and PfATP6, a parasite-encoded sarcoplasmic–endoplasmic reticulum calcium ATPase. Briefly, the mechanism of action of artemisinin appears to involve two steps. In the first step (activation), intra-parasitic iron catalyses the cleavage of the endoperoxide bridge and the generation of free radicals. In the second step (alkylation), the artemisinin-derived free radicals form covalent bonds with parasite proteins.

An endoperoxide bridge lies at the heart of the antiparasitic activity of artemisinin, although the chemical nature of the interactions between artemisinins (particularly the essential endoperoxide) and parasite target(s) is not well understood. The cleavage of the endoperoxide moiety leads to the formation of reactive oxygen species (ROS) and carbon-centred radicals. These highly reactive molecules target several proteins in Plasmodia, which is thought to result in the killing of the microorganisms. The role of ferrous species in the antimalarial actions of artemisinins is also debated, because these cations can catalyse *in vitro* reactions of some artemisinins, including their decomposition in aqueous solutions [40].

Besides artemisinin, other sesquiterpene lactones isolated from *Artemisia* species also showed antimalarial properties. Leaves and flowers of *A. gorgonum* were phytochemically investigated and resulted in the isolation and characterization of several sesquiterpene lactones, 11 guaianolides, 1 secoguaianolide and 2 germacranolides [34]. Most compounds exhibited modest antiplasmodial activities, with ridentin, Fig. 8, and hanphyllin, Fig. 9, being the most interesting compounds, with an inhibitory concentration 50 (IC_{50}) of 5.4 and 2.3μg/ml against *P. falciparum*, respectively. The antimalarial activity of these compounds may be attributed to the exomethylene group of the

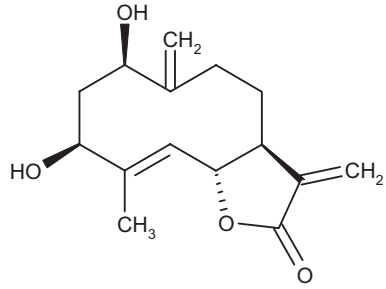


FIGURE 8 Structure of ridentin.

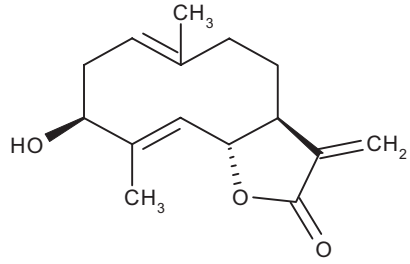


FIGURE 9 Structure of hanphyllin.

lactone function, which is an essential group for biological activities of sesquiterpene lactones.

Besides its use in the treatment of malaria, the bioactivity of artemisinin and its derivatives is much broader. These compounds have also been submitted to studies aimed at exploring other uses for this drug class.

Artemisinins are active against other parasite species *in vitro*, including protozoa that are phylogenetically unrelated to apicomplexan parasites such as the *Plasmodium* species that cause malaria, particularly *Leishmania*. Visceral leishmaniasis, caused by the protozoan *Leishmania* sp., affects 500,000 people annually, with the Indian subcontinent contributing a significant proportion of these cases. Emerging refractoriness to conventional antimony therapy has highlighted the need for safer yet effective antileishmanial drugs. Artemisinin demonstrated antipromastigote activity with IC_{50} ranging from 100 to 120μM in the *Leishmania* species studied (*Leishmania donovani*, *Leishmania infantum*, *Leishmania tropica*, *Leishmania mexicana*, *Leishmania amazonensis* and *Leishmania braziliensis*) [41]. *L. donovani*-infected macrophages demonstrated decreased production of nitrite as well as mRNA expression of inducible nitric oxide synthase (iNOS), which was normalised by artemisinin, indicating that it exerted both a direct parasiticidal activity as well as inducing a host protective response. For *in vivo* studies, the BALB/c mouse model meets eligibility requirements such as the chronic infection pattern, which resembles human visceral leishmaniasis. In these studies, treatment with artemisinin led to a

significant reduction in splenic weight, which was accompanied by a significant inhibition of parasites and a restoration of cytokines such as interferon-γ and interleukin-2 (IL-2). These findings have delineated the therapeutic potential of artemisinin in experimental visceral leishmaniasis. In addition, and as shown in recent years, its potential application includes the treatment of infections from several viruses such as human cytomegalovirus and other members of the Herpesviridae family (e.g., herpes simplex virus type 1 and Epstein-Barr virus), hepatitis B and C virus, and bovine viral diarrhoea virus [42–44], infections from the insects *Epilachna paenulata* and *Spodoptera eridania* [45], the parasite *Eimeria tenella* [46] and from *Haemonchus contortus*, a blood-sucking abomasal parasite of small ruminants which is responsible for major losses to producers worldwide [47].

Artemisinin is not the only medicinal compound in *Artemisia* species. The genus is a rich source of other sesquiterpene lactones that might also have similar properties, with low risk of mammalian toxicity. (+)-Santonide, Fig. 10, is an anthelmintic sesquiterpenoid keto lactone, obtained from various Asia Minor species of *Artemisia*, that has recently aroused considerable pharmaceutical interest [48].

Chung *et al*. [31] investigated the antibacterial activities of vulgarone B, Fig. 6, a component of *A. iwayomogi*, against some antibiotic susceptible and resistant human pathogens. These results demonstrated that vulgarone B may be a promising candidate for a safe, effective and natural agent against antibiotic-resistant *Staphylococcus aureus*. The antibiotic mechanism involved might be related to DNA cleavage. This compound is also isolated as an active antifungal constituent from *A. douglasiana* [32]. Vulgarone B showed antifungal activity against *Colletotrichum acutatum*, *Colletotrichum fragariae*, *Colletotrichum gloesporioides* and *Botrytis cinerea*. For the studies of structure–activity relationship, it is evident that the α,β-unsaturated carbonyl group is essential for the antifungal activity of vulgarone B, postulating that one of the possible modes of action of this antifungal compound may be due to its role as a Michael-type acceptor for biological nucleophiles. Vulgarone B is

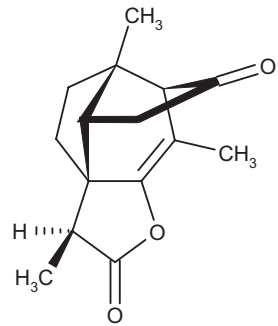


FIGURE 10 Structure of (+)-santonide.

not the only medicinal sesquiterpene lactone from *A. douglasiana*. Dehydroleucodine is a sesquiterpene lactone of the guaianolide type isolated from this plant, which presented antimicrobial activity against *Helicobacter pylori*, the major cause of chronic gastritis and peptic ulcer, and an important factor in the pathogenesis of gastric cancer [33].

Examples of other antimicrobial sesquiterpene lactones isolated from *Artemisia* genus also included seco-tanapartholides from *A. princeps*, which showed growth-inhibitory effects on human intestinal bacteria [49].

Sesquiterpene lactones from *Artemisia* genus have also been submitted to studies aimed at exploring other uses for this drug class. Their antidisease properties include potent anti-inflammatory and anticancer activity in *in vitro* studies and in *in vivo* models. These investigations are discussed in more detail here.

ANTI-INFLAMMATORY AND IMMUNOSUPPRESSIVE ACTIVITIES

The inflammatory process plays a key role in innate and acquired immune function. Low-grade inflammation is also involved in the ethiology of cardiovascular diseases, in inflammatory diseases such as arthritis, and in allergies such as asthma. There are several reports that various mediators, including nitric oxide (NO), prostaglandins (PGs) and cytokines, participate in inflammatory events. Inflammatory cytokines are signalling proteins and are expressed in a number of tissues, notably monocytes/macrophages, vascular endothelial cells, adipose tissue and neurons, which respond to injury or infection. iNOS and cyclooxygenase-2 (COX-2) catalyse the formation of NO and PGs, respectively. They are induced by cytokines or other inflammatory stimuli such as bacterial lipopolysaccharide (LPS) and infectious pathogens. Nuclear transcription factor kappa B (NF-κB) regulates various genes involved in immune and acute phase inflammatory responses. NF-κB activation, in response to pro-inflammatory stimuli, involves the rapid phosphorylation of inhibitors of NF-κB (IκB) by the IκB kinase (IKK) signalosome complex. Free NF-κB produced by this process translocates to the nucleus, where it binds to κB-binding sites in the promoter regions of target genes. It then induces the transcription of pro-inflammatory mediators such as iNOS, COX-2, tumour necrosis factor-α (TNF-α), IL-1β, IL-6 and IL-8 [50]. The mitogen-activated protein kinase also regulates inflammatory and immune responses, and their signalling pathways are involved in LPS-induced COX-2 and iNOS expression in macrophages.

A review of the literature concerning the evaluation of artemisinin and other sesquiterpene lactones from the *Artemisia* genus reveals that many studies into their *in vitro* and *in vivo* anti-inflammatory and immunosuppressive activities have been carried out in recent years. Some of these compounds were isolated by bioassay-guided fractionation, after previously detecting activity on the part of the plant.

In *in vivo* experiments, artemisinin, Fig. 1, was able to suppress delayed-type hypersensitivity against sheep blood capsule in Balb/c mice, suggesting it as an immunosuppressive agent [51]. It is probable that artemisinin leads to a decrease in IL-2 and its receptors and probably blocks IL-2 activity. Additionally, artemisinin inhibits iNOS and NF-κB activation [52].

Guardia *et al*. [53] investigated the effect of dehydroleucodine isolated from *A. douglasiana* in two experimental models: arthritis induced by Freund's adjuvant carrageenan and cotton pellet-induced granuloma. This sesquiterpene lactone inhibited both chronic and acute adjuvant carrageenan-induced inflammation phases, and was most effective on the chronic phase. In the granuloma test, the compound also inhibited inflammation. It is suggested that the anti-inflammatory activity of dehydroleucodine may be attributed to interference with multiple targets on the level of transcription factors, such as NF-κB and cytokines.

Artemisolide, Fig. 2, was isolated as an NF-κB inhibitor from *A. asiatica* by activity-guided fractionation [54,55]. This compound inhibited NF-κB transcriptional activity in LPS-stimulated macrophages RAW 264.7, with an IC_{50} value of 5.8μM. The compound was also effective in blocking NF-κB transcriptional activities elicited by the expression vector encoding the NF-κB p65 or p50 subunits, bypassing the inhibitory κB degradation signalling NF-κB activation. This compound, artemisolide, together with several sesquiterpene lactones such as 3α,4α-epoxyrupicolins C–E, 3-methoxytanapartholide, deacetyllaurebiolide, moxartenolide and arteminolides B and D, Fig. 3, were also isolated by bioassay-guided fractionation from the methanol extract of the aerial parts of *A. sylvatica*, using the NF-κB mediated reporter gene assay [25]. All isolated compounds displayed inhibitory activity on the LPS-induced NF-κB activation, and NO and TNF-α production. Several studies have investigated the functional Michael acceptors of some sesquiterpene lactones, such as an exomethylene group conjugated to a carbonyl group, which can react with biological nucleophiles, especially the sulfhydryl group of the cysteine residue in the proteins, which plays an important role in the NF-κB activation and DNA binding process [56]. All sesquiterpene lactones included in this study contain an α-methylene-γ-lactone ring as a common functional group. Arteminolide B and D have three more possible Michael acceptors other than α-methylene-γ-lactone ring, suggesting that the more Michael acceptors in the structure, the stronger the NF-κB inhibitory activity. Furthermore, among the new compounds, 3α,4α-epoxyrupicolins C–E, C, Fig. 11, showed the most potent effect, suggesting that the angeloyloxy group at C-8 may contribute to NF-κB inhibition.

Examples of other anti-inflammatory sesquiterpene lactones from the *Artemisia* genus also included dimeric guaianolides from *A. anomala* [26], and those isolated from *Artemisia khorassanica* Podl., which inhibits iNOS and COX-2 expression through the inactivation of NF-κB [57].

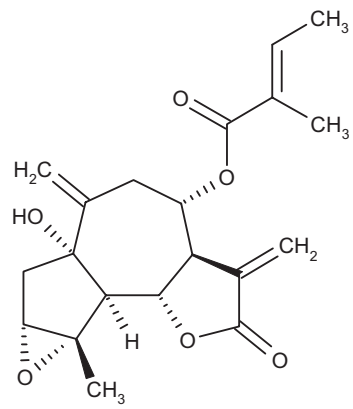


FIGURE 11 Structure of 3α,4α-epoxyrupicolin C.

ANTITUMOUR ACTIVITY

Carcinogenesis can be viewed as a multistage, microevolutionary process. It is generally agreed that tumours can derive from single abnormal cells, and work with experimental systems shows that carcinogenesis can be divided into three major stages: initiation, promotion and progression. Initiation is a heritable aberration of a cell, which appears to be irreversible and can result from DNA damage. Cells so initiated can undergo transformation to malignancy if promotion and progression follow. Promotion is partly reversible and accounts for a major portion of the lengthy latent period of carcinogenesis. The final stage of tumour formation is the progression of a benign growth to a malignant neoplasm. There is low growth control, an escape from the host's defence mechanism and metastasis. Because conventional treatments such as surgical resection, radiation therapy and chemotherapy are still not satisfactory, prevention of this disease or at least stopping it at its inception is important.

Plants have been demonstrated to be a very viable source of clinically relevant anticancer compounds. However, ethnopharmacological information has been poorly utilized in the past in the search for new principles against cancer. In many ethnomedical systems, reports of specific antitumour uses of plants are rarely found, mainly because cancer is a disease that involves a complex set of signs and symptoms. Considering these observations, a set of ethnobotanical usages such as immune and skin diseases, inflammation, infections, parasitic and viral diseases can be taken into account because they reflect disease states bearing relevance to cancer or a cancer symptom.

A review of the literature concerning the evaluation of artemisinin, Fig. 1, and other sesquiterpene lactones from the *Artemisia* genus reveals that there have been many studies into their antineoplastic activities in recent years [58]. Their antidisease properties include potent anticancer activity in *in vitro* studies and in *in vivo* models of leukaemia, melanoma, breast, ovarian, lung, prostate

and renal cancer cell lines. The pleiotropic response in cancer cells includes growth inhibition by cell cycle arrest, apoptosis, inhibition of angiogenesis, disruption of cell migration and modulation of nuclear receptor responsiveness [59]. These effects of artemisinin and its derivatives result from perturbation of many cellular signalling pathways, although little is known about the molecular mechanisms of this response. However, the mechanism of its antitumour effect is its focus on the endoperoxide bridge structure, which reacts with a ferrous iron atom to form free radicals leading directly to cellular destruction. In addition, with the knowledge of a high accumulation of iron in cancer cells, the researchers have been interested in the inhibition of calmodulin by artemisinin as a possible method for controlling the activity of malignant cells. The investigations showed that artemisinin can inhibit the calmodulin-mediated activation of phosphodiesterase [60].

Artemisinin induces cell growth arrest in the human melanoma cell lines A375M and A375P, and affects cells viability with cytotoxic and growth-inhibitory effects, while it was not effective in contrasting proliferation of other tumour cell lines such as the human breast adenocarcinoma cell lines MCF7 and MKN [61]. In addition, artemisinin affected the migratory ability of A375M cells by reducing metalloproteinase-2 production and down-regulating α-V-β-3-integrin expression. These findings introduce a potential of artemisinin as a chemotherapeutic agent in melanoma treatment.

Willoughby *et al*. [62] reported that artemisinin treatment triggers a stringent G1 cell cycle arrest of lymph node carcinoma of the prostate LNCaP, human prostate cancer cell, accompanied by a rapid down-regulation of cyclin-dependent kinase-2 (CDK2) and CDK4 protein and transcript levels. Transient transfection with promoter-linked luciferase reporter plasmids revealed that artemisinin strongly inhibits CDK2 and CDK4 promoter activity. Deletion analysis of the CDK4 promoter revealed a 231-bp artemisinin-responsive region between −1737 and −1506. Site-specific mutations revealed that the Sp1 transcription factor site at −1531 was necessary for artemisinin responsiveness in the context of the CDK4 promoter. Taken together, these results demonstrated that a key event in the artemisinin antiproliferative effects in prostate cancer cells is the transcriptional down-regulation of CDK4 expression by disruption of Sp1 interactions with the CDK4 promoter. These results suggest that the antiproliferative effects of artemisinin on prostate cancer cells, and likely other cancer cell types, are independent of its oxidative potential.

Nam *et al*. [63] investigated the antitumour activity of artemisinin and its various derivatives (dihydroartemisinin, dihydroartemisinin 12-benzoate, 12-(2'-hydroxyethyl) deoxoartemisinin, 12-(2'-ethylthio) deoxoartemisinin dimer and deoxoartemisinin trimer) on the oral cancer cell line YD-10B. The deoxoartemisinin trimer was found to have greater antitumour effect on tumour cells than other commonly used chemotherapeutic drugs such as 5-fluorouracil, cisplatin and paclitaxel. Furthermore, the ability of artemisinin and its derivatives to induce apoptosis highlights their potential as chemotherapeutic agents,

as many anticancer drugs achieve their antitumour effects by inducing apoptosis in tumour cells. In addition, Stockwin *et al*. [64] investigated two dimeric artemisinins to determine their mechanism of action. Dimers were 1000-fold more active than monomers, and treatment was associated with increased ROS and apoptosis induction. These results highlight the importance of iron, haem and ROS in activity. Microarray analysis of dimer treated cells identified DNA damage, iron/haem and cysteine/methionine metabolism, antioxidant response and endoplasmic reticulum stress as affected pathways, implicating indirect endoplasmic reticulum-stress induction as a central mechanism of artemisinin dimer activity.

One of these semi-synthetic derivatives of artemisinin, dihydroartemisinin, showed apoptosis activity in cultured human lung cancer cells SPC-A-1 [65]. These results demonstrated that calcium and survivin participated in the apoptotic signalling pathways. The observations also indicated that dihydroartemisinin down-regulated the mRNA and protein expression level of survivin in SPC-A-1, whereas it did not affect those of caspase-4. However, Lu *et al*. [66] demonstrated that dihydroartemisinin induces caspase-3-dependent apoptosis in human lung adenocarcinoma ASTC-a-1 cells, in a dose- and time-dependent manner, which was accompanied by mitochondrial morphology changes, the loss of mitochondrial transmembrane potential and the activation of caspase-3. However, the cellular targets and molecular mechanism of dihydroartemisinin-induced apoptosis is poorly defined. More recently, Lu *et al*. [67] investigated the roles of dihydroartemisinin-elicited ROS in the dihydroartemisinin-induced activation of Bcl-2 family proteins, mitochondrial dysfunction, caspase cascade and cell death. These findings demonstrate for the first time that dihydroartemisinin induces cell apoptosis by triggering ROS-mediated caspase-8/Bid activation and the mitochondrial pathway, which provides some novel insights into the application of this compound as a potential anticancer drug and a new therapeutic strategy by targeting ROS signalling in lung adenocarcinoma therapy in the future.

Zhou *et al*. [68] investigated the inhibitory effect of artesunate, another semi-synthetic derivative of artemisinin, on angiogenesis and on vascular endothelial growth factor (VEGF) production in chronic myeloid leukaemic K562 cells *in vitro* and *in vivo*. VEGF is a multifunctional cytokine that acts as both a potent inducer of vascular permeability and a specific endothelial cell mitogen. VEGF is commonly expressed in a wide variety of human tumour cells and has been associated with angiogenesis, growth, metastasis and poor outcome in solid tumours. The results showed that artesunate could decrease the VEGF level in K562 cells, even at a lower concentration (2μM/l). The antiangiogenic effect of artesunate was further evaluated *in vivo* in a chicken chorioallantoic membrane neovascularisation model. The results indicate that the stimulating angiogenic activity was decreased in response to the K562 cells treated or pretreated with artesunate in a dose-dependent manner (3–12μM/l). These findings suggest that artesunate might have a potential antileukemia effect as a treatment

for chronic myeloid leukaemia therapy, or as an adjuvant to standard chemotherapy regimens. Besides the inhibitory effect of artesunate on VEGF expression, which might contribute to its antiproliferative effect in K562 cells, several studies suggest that DNA damage induced by artesunate contributes to its therapeutic effect against cancer cells [69]. This compound also reduces chicken chorioallantoic membrane neovascularisation and exhibits antiangiogenic and apoptotic activity on human microvascular dermal endothelial cells [70]. These results suggest that the antiangiogenic effect induced by artesunate might occur by causing cellular apoptosis. These findings, and the known low toxicity, indicated that artesunate might be a promising candidate for angiogenesis inhibitors. Other studies also demonstrated that NO generation and signalling play a role in exhibiting cytotoxic activity of artesunate, pointing to a multifactorial mode of action of this compound [71].

The activities of artemisinin and derivatives are mediated chemically by α,β-unsaturated carbonyl structures, such as an α-methylene-γ-lactone, an α,β-unsaturated cyclopentenone or a conjugated ester. These structure elements react with nucleophiles, especially cysteine sulphydryl groups, by a Michael-type addition. Therefore, exposed thiol groups, such as cysteine residues in proteins, appear to be the primary targets of sesquiterpene lactones. The differences in activity between individual sesquiterpene lactones may be explained by different numbers of alkylating structural elements. However, other factors such as lipophilicity, molecular geometry and the chemical environment of the target sulphydryl, may also influence the activity of sesquiterpene lactones.

Other anticancer sesquiterpene lactones isolated from the *Artemisia* genus also included arteminolides A–D, Fig. 3, isolated from the aerial parts of *A. argyi* [24]. Arteminolides were reported to have inhibitory activity on farnesyl-protein transferase (FPTase), with IC_{50} values of 0.7–1μM. FPTase inhibitors have been shown to inhibit the growth of human tumours in mouse xenograft models and, more dramatically, in transgenic mouse models. Specific inhibitors of FPTase might be interesting chemical leads to develop effective therapeutic agents for the treatment of cancer. To prove the antitumour effects of arteminolides, potent FPTase inhibitors, investigations have been carried out into tumour growth assay against human cancer cells and in human tumour xenograft. It is well known that the exomethylene group on the sesquiterpene lactone is an essential group for biological activities, including cytotoxicity against tumour cells. Chemical studies showed that various cytotoxic sesquiterpene lactones react with thiols such as cysteine by rapid Michael-type addition. It is generally accepted that the mechanism of cytotoxicity is due to the alkylation of biological nucleophiles in the α,β-unsaturated carbonyl moiety. Arteminolides A–D have very similar antitumour profiles against the tested human tumour cell lines, and the only structural differences among them are acyl groups at C-8. Therefore, the results suggest that the acyl side chains at C-8 would not significantly contribute antitumour effects. To see whether the α-methylene-γ-lactone group of arteminolides was involved in the inhibition of tumour cell growth,

hydrogenation of arteminolides B and C was performed. The low antiproliferative activity of hydrogenated arteminolides indicates that the α-methylene-γ-lactone group of sesquiterpene lactones significantly contributed to the growth inhibition of tumour cells. Antitumour activity of arteminolide C in human tumour xenografted nude mice studies of arteminolides supports the conclusion that these compounds would be good lead molecules for the development of antitumour drugs. This species, *A. argyi*, also yielded the sesquiterpene lactone artemisolide, Fig. 2, which exhibited *in vitro* cytotoxic activity against cancer cell lines (SW620 colon, human promyelocytic leukaemia HL-60 and human acute lymphoblastic leukaemia Molt-4) [23].

Examples of other antitumour sesquiterpene lactones from the *Artemisia* genus also included dimeric guaianolides from *A. anomala* [26], and artemdubolides A–H, highly oxygenated guaianolides isolated from *A. dubia*, which showed weak cell growth inhibition of the human colon carcinoma Colo205 and human melanoma MDA-MB-435 cells [28].

ACKNOWLEDGEMENT

The technical assistance of Ms. Brooke-Turner is gratefully acknowledged.

ABBREVIATIONS

ACTS	artemisinin-based combinatory therapies
ROS	reactive oxygen species
IC_{50}	inhibitory concentration 50
iNOS	inducible nitric oxide synthase
IL	interleukins
NO	nitric oxide
PGs	prostaglandins
COX-2	cyclooxygenase-2
LPS	lipopolysaccharide
NF-κB	nuclear factor kappa B
IκB	inhibitors of NF-κB
IKK	IκB kinase
TNF-α	tumour necrosis factor-α
CCK	cyclin-dependent kinase
VEGF	vascular endothelial growth factor
FPTase	farnesyl-protein transferase

REFERENCES

[1] M. Willcox, J. Alternative Complement. Med. 15 (2009) 101–109.
[2] E. Hsu, Br. J. Clin. Pharmacol. 61 (2006) 666–670.
[3] S. Krishna, L. Bustamante, R.K. Haynes, H.M. Staines, Trends Pharmacol. Sci. 29 (2008) 520–527.
[4] N.J. White, Science 320 (2008) 330–334.

[5] S. De Ridder, F. Van der Kooy, R. Verpoorte, J. Ethnopharmacol. 120 (2008) 302–314.
[6] T. Kuhn, Y. Wang, Prog. Drug Res. 66 (2008) 385–422.
[7] T. Baytop, Therapy with Medicinal Plants in Turkey, Istanbul University Press, Istanbul, Turkey, 1984.
[8] D.L. Klayman, Science 228 (1985) 1049–1055.
[9] D. Bramwell, Z.I. Bramwell (Eds.), Flores silvestres de las Islas Canarias, Rueda, Madrid, Spain, 1990.
[10] K. Otsuka, J. Shoji, M. Takido, S. Cho, A Pictorial Encyclopedia of Chinese Medical Herbs, Chnokoron-Sha Inc., Tokyo, Japan, 1992.
[11] T.T. Hien, N.J. White, Lancet 341 (1993) 603–608.
[12] V. Ligaa, Medicinal Plants of Mongolia Used in Mongolian Traditional Medicine. RSA, Seoul, South Korea, 1996.
[13] A. Zargari, Iranian Medicinal Plants, Tehran University Publications, Tehran, Iran, 1997.
[14] D. Moermann, Native American Ethnobotany, Timber Press Inc., Portland, USA, 1998.
[15] P. Perez de Paz, C. Hernández Padrón, In: S.L. Francisco Lemus (Ed.), Plantas medicinales o útiles en la Flora Canaria. Aplicaciones populares, La Laguna, Spain, 1999.
[16] J. Gruenwald, PDR for Herbal Medicines, Montvale, USA, 2000.
[17] P. Baker, The Book of Absinthe: A Cultural History, Grove Press, New York, USA, 2001.
[18] M. Ballero, F. Poli, G. Sachetti, M.C. Loi, Fitoterapia 72 (2001) 788–801.
[19] M. Tadesse, Flora of Ethiopia and Eritrea, Uppsala University, Addis Abeba, Ethiopia, 2004.
[20] A.J. Skyles, B.V. Sweet, Am. J. Health Syst. Pharm. 61 (2004) 239–242.
[21] N. Liu, F. Van der Kooy, R. Verpoorte, S. Afr. J. Bot. 75 (2009) 185–195.
[22] A. Singh, R. Sarin, Bangladesh J. Pharmacol. 5 (2010) 17–20.
[23] J.H. Kim, H.K. Kim, S.B. Jeon, K.H. Son, E.H. Kim, S.K. Kang, N.D. Sung, B.M. Kwon Tetrahedron Lett. 43 (2002) 6205–6208.
[24] S.H. Lee, M.Y. Lee, H.M. Kang, D.C. Hun, K.H. Son, D.C. Yong, N.D. Sung, C.W. Lee, H.M. Kim, B.M. Kwon, Bioorg. Med. Chem. 11 (2003) 4545–4549.
[25] H.Z. Jin, J.H. Lee,., D. Lee, Y.S. Hong, Y.H. Kim, J.J. Lee, Phytochemistry 65 (2004) 2247–2253.
[26] J. Wen, H. Shi, Z. Xu, H. Chang, C. Jia, K. Zan, Y. Jiang, P. Tu, J. Nat. Prod. 73 (2010) 67–70.
[27] K. Zan, X.Q. Chen, Q. Fu, S.P. Shi, S.X. Zhou, M.T. Xiao, P.F. Tu, Biochem. Syst. Ecol. 38 (2010) 431–434.
[28] Z.S. Huang, Y.H. Pei, C.M. Liu, S. Lin, J. Tang, D.S. Huang, T.F. Song, L.H. Lu, Y.P. Gao, W.D. Zhang, Nat. Prod. Chem. 76 (2010) 1710–1716.
[29] A.A. Ahmed, S.A. El Moghazy, M.A. El Shanawany, H.F. Abdel-Ghani, J. Karchesy, G. Sturtz, K. Dally, P.W. Pare, J. Nat. Prod. 67 (2004) 1705–1710.
[30] K.H. Lee, Y.D. Min, S.Z. Choi, H.C. Kwon, O.R. Cho, K.C. Lee, R.R. Lee, Arch. Pharm. Res. 27 (2004) 1016–1019.
[31] E.Y. Chung, Y.H. Byun, E.J. Shin, H.S. Chung, Y.H. Lee, S. Shin, Arch. Pharm. Res. 32 (2009) 1711–1719.
[32] K.M. Meepagala, J.M. Kuhajek, G.D. Sturtz, D.E. Wedge, J. Chem. Ecol. 29 (2003) 1771–1780.
[33] A.E. Vega, G.H. Wendel, A.O.M. Maria, L. Pelzer, J. Ethnopharmacol. 124 (2009) 653–655.
[34] R. Ortet, S. Prado, E. Mouray, O.P. Thomas, Phytochemistry 69 (2008) 2961–2965.
[35] S. Kebede, S. Duales, A. Yokovide, W. Aleman, East Afr. J. Public Health 7 (2010) 20–29.
[36] T. Bousema, C. Drakeley, Clin. Microbiol. Rev. 24 (2011) 377–410.
[37] C. McNamara, E.A. Winzeler, Future Microbiol. 6 (2011) 693–704.
[38] G.D. Brown, Molecules 15 (2010) 7603–7698.

[39] L. Cui, X.Z. Su, Expert. Rev. Anti-Infect. Ther. 7 (2009) 999–1013.
[40] R.K. Haynes, W.C. Chan, C.M. Lung, A.C. Uhleman, V. Eckstein, D. Taramelli, S. Parapini, D. Monti, S. Krishna, Chem. Med. Chem. 2 (2007) 1480–1497.
[41] R. Sen, S. Ganguly, P. Saha, M. Chatterjee, Int. J. Antimicrob. Agents 36 (2010) 43–49.
[42] M.R. Romero, T. Efferth, M.A. Serrano, B. Castano, R.I. Macias, O. Briz, J.A. Marin, Antiviral Res. 68 (2005) 75–83.
[43] M.R. Romero, M.A. Serrano, M. Vallejo, T. Efferth, M. Alvarez, J.J. Marín, Planta Med. 72 (2006) 1169–1174.
[44] T. Efferth, M.R. Romero, D.G. Wolf, T. Stamminger, J.J. Marin, M. Marschall, Clin. Infect. Dis. 47 (2008) 804–811.
[45] M.E. Maggi, A. Mangeaud, M.C. Carpinella, C.G. Ferrayoli, G.R. Valladares, S.M. Palacios, J. Chem. Ecol. 31 (2005) 1527–1536.
[46] E. Del Cacho, M. Gallego, M. Francesh, J. Quilez, C. Sánchez-Acedo, Parasitol. Int. 59 (2010) 506–511.
[47] J.M. Squires, J.F. Ferreira, D.S. Lindsay, A.M. Zajac, Vet. Parasitol. 175 (2011) 103–108.
[48] J. Zinczuk, E.A. Ruveda, H.W. Thompson, R.A. Lalancette, Acta Crystallogr. 63 (2007) 1490–1491.
[49] S.H. Cho, Y.E. Na, Y.J. Ahn, J. Appl. Microbiol. 95 (2003) 7–12.
[50] S. Ghosh, M.S. Hayden, Nat. Rev. Immunol. 8 (2008) 837–848.
[51] S. Noori, G.A. Naderi, Z.M. Hassan, Z. Habibi, S.Z. Bathaic, S.M. Hashemi, Int. Immunopharmacol. 4 (2004) 1301–1306.
[52] E. Aldieri, D. Atragene, L. Bergandi, C. Riganti, C. Costamagno, A. Bosia, D. Ghigo, FEBS Lett. 552 (2003) 141–144.
[53] T. Guardia, A.O. Juarez, E. Guerreiro, J.A. Guzmán, L. Pelzer, J. Ethnopharmacol. 88 (2003) 195–198.
[54] A.M. Reddy, J.Y. Lee, J.H. Seo, B.H. Kim, E.Y. Chung, S.Y. Ryu, Y.S Kim, C.K. Lee, K.R. Min, Y. Kim, Arch. Pharm. Res. 29 (2006) 591–597.
[55] B.H. Kim, J.Y. Lee, J.H. Seo, H.Y. Lee, S.Y. Ryu, B.W. Ahn, C.K. Lee, B.Y. Hwuang, S.B. Han, Y. Kim, Biochem. Biophys. Res. Commun. 361 (2007) 593–598.
[56] P. Rungeler, V. Castro, G. Mora, N. Goren, W. Vichnewski, H.L. Pahl, I. Merfort, T.J. Schmidt, Bioorg. Med. Chem. 7 (1999) 2343–2352.
[57] S.A. Emami, S.Z. Taghizadeh Rabe, M. Iranshashi, A. Ahí, M. Mohmoudi, Immunopharmacol. Immunotoxicol. 32 (2010) 688–695.
[58] T. Efferth, Planta Med. 73 (2007) 299–309.
[59] G.L. Firestone, S.N. Sundar, Expert. Rev. Mol. Med. 11 (2009) 32.
[60] S. Noori, Z.M. Hassan, B. Rezai, A. Rustaiyan, Z. Habibi, F. Fallahian, Int. Immunopharmacol. 8 (2006) 1744–1747.
[61] E. Buommino, A. Baroni, N. Canozo, M. Petrazzuolo, R. Nicoletti, A. Vozza, M.A. Tufano, Invest. New Drugs 27 (2009) 412–418.
[62] J.A. Willoughby Sr., S.N. Sundar, M. Cheung, A.S. Tin, J. Modiano, G.L. Firestone, J. Biol. Chem. 284 (2009) 2203–2213.
[63] W. Nam, J. Tak, J.R. Ryu, M. Jung, J.I. Yook, H.J. Kim, I.H. Cha, Head Neck 29 (2007) 335–340.
[64] L.H. Stockwin, B. Han, S.X. Yu, M.G. Hollingshead, M.A. Elsohly, W. Gul, et al., Int. J. Cancer 125 (2009) 1266–1275.
[65] D. Mu, W. Chen, B. Yu, C. Zhang, Y. Zhang, H. Qi, Meth. Find. Exp. Clin. Pharmacol. 29 (2007) 33–38.
[66] Y.Y. Lu, T.S. Chen, J.L. Qu, W.L. Pan, L. Sun, X.B. Wei, J. Biomed. Sci. 2 (2009) 16.

[67] Y.Y. Lu, T.S. Chen, X.P. Wang, L. Li, J. Biomed. Opt. 15 (2010) 1–16.
[68] H.J. Zhou, W.Q. Swang, G.D. Wu, J. Lee, A. Li, Vascul. Pharmacol. 47 (2007) 131–138.
[69] P.C. Li, E. Lam, W.P. Roos, M.Z. Zdzienicka, B. Kaina, Cancer Res. 68 (2008) 4347–4351.
[70] H.L. Huan, L.Y. Li, B.L. Shang, Cancer Lett. 211 (2004) 163–173.
[71] V.B. Konkimalla, M. Blunder, B. Korn, S.A. Soomro, H. Jansen, W. Chang, G.H. Posner, R. Bauer, T. Efferth, Nitric Oxide 19 (2008) 184–191.

Chapter 3

Secondary Metabolites with Antinematodal Activity from Higher Plants

Ligang Zhou[†], Jingguo Wang[*], Kui Wang[†], Jianmei Xu[†], Jianglin Zhao[†], Tijiang Shan[†] and Chao Luo[†]

[†]Department of Plant Pathology, College of Agronomy and Biotechnology, China Agricultural University, Beijing, China

[*]Department of Plant Nutrition, College of Resources and Environmental Sciences, China Agricultural University, Beijing, China

INTRODUCTION

Plant-parasitic nematodes cause significant economic losses in a wide variety of crops [1]. They may reduce crop yield through direct cell destruction, vectoring viruses, or indirectly by facilitating invasion of fungi and bacteria through feeding and movement across the roots. Moreover, crop losses could be more extensive if disease complexes are exacerbated by nematodes and other soilborne pathogens. Crop losses may also be more severe in tropical and subtropical regions where nematode reproduction rates are generally much higher than in temperate zones [2].

Plant-parasitic nematodes may be controlled by fumigants and other chemical nematicides, rotating with non-host crops, and the use of resistant cultivars. However, nematicides cannot provide long-term suppression of nematodes, and the growing concerns over environment and human health are resulting in increased restrictions on their use [3]. Furthermore, the wide host range of some nematode species and the unavailability of resistant varieties limit the use of crop rotation in several production systems [4]. Some safe strategies for nematode control have been developed based on biological control agents and organic amendments. One such alternative is to explore naturally occurring compounds in plants, which are known as plant secondary metabolites. Other alternatives include antinematodal agents from microorganisms and animals [5,6].

Bioactive Natural Products (Part Q), Vol. 37. http://dx.doi.org/10.1016/B978-0-444-59514-0.00003-1

The ability of some plants to exert antinematodal (or nematocidal, or nematostatic) activity has been known for a long time. Excellent general reviews on cultivation tactics for nematode control [4,7–9], plant extracts [10–12] including essential oils [13–20] on their antinematodal activity have been published. Although experiments with crude preparations are valuable for development of biologically based control strategies, these experiments are usually difficult to interpret biologically or biochemically. For example, toxicants, nutrients and phytohormones in such extracts may act directly upon plant hosts. Specific compounds within such extracts may act synergistically or antagonistically [22].

There were no detailed reports to concentrate on the antinematodal metabolites from higher plants though a few related reviews have been published in the past 20 years [10,11,21–23]. In this article, we review the information available on antinematodal secondary metabolites from higher plants. These antinematodal metabolites from plants were grouped according to their biosynthetic origin, often presumed, to facilitate the comparison between structure and activity. This article does not concentrate on the studies in which plants or plant crude extracts including essential oils were examined for nematode-antagonistic activity, unless the active compound was identified.

ALIPHATICS

Aliphatics (or called aliphatic compounds) are the common constituents from plants and animals, and usually exist *in vivo* as esters. Extracts from the roots of *Iris japonica* (Iridaceae) showed their antinematodal activity towards the rice white-tip nematode (*Aphelenchoides besseyi*). The main active metabolites were found to reside in the acidic portion of the extract, and were further identified as palmitic acid (**1**), oleic acid (**2**), linoleic acid (**3**), 2-undecylenic acid (**4**), and myristic acid (**5**) [24]. Butyric acid (**6**) found in decomposed rye (*Secale cereale*, Graminae) and timothy (*Phleum pretense*, Graminae) was screened to exhibit antinematodal activity on *Meloidogyne incognita* and *Pratylenchus penetrans* at 880μg/ml [25].

The methanol extract of *Melia azedarach* (Meliaceae) showed its nematicidal activity against *M. incognita*. The main active constituents were butyric acid (**6**), acetic acid (**7**), and hexanoic acid (**8**), which were tested individually for nematicidal activity against the second-stage juveniles [26].

1-Octanol (**9**), an aliphatic alcohol, was successfully isolated from the methanol extract of the whole plant of *Allium grayi* (Liliaceae) *via* the bioassay against *M. incognita* [27].

Di-*n*-butyl succinate (**10**), which was an artefact probably, obtained from peanut (*Arachis hypogaea*, Leguminosae) was found to be active against *Pratylenchus coffeae* at 100μg/ml. Eleven other nematicidal dialkyl succinates were also obtained from this plant [28].

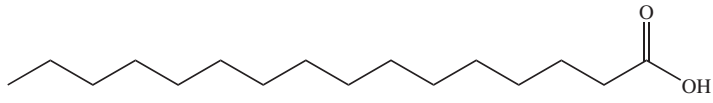
A nematicidal triglyceride namely *sn*-glycerol-1-eicosa-9,12-dienoate-2-palmitoleate-3-linoleate (**11**) was isolated from the seeds of *Argemone*

mexicana (Papaveraceae). Its median lethal concentration (LC_{50}) against *M. incognita* juveniles *in vivo* was 90μg/ml. When triglyceride (**11**) was treated at 100μg/ml, it prevented subsequent infection of *M. incognita* juveniles to tomato plants [29].

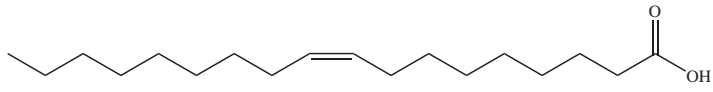
Two aliphatic acid esters namely methyl pelargonate (**12**) and ethylene glycol pelargonate (**13**) reduced galling of tomato roots infected with *Meloidogyne javanica*. In greenhouse tests, methyl pelargonate (**12**) at 1.6mg/l was effective against *Heterodera glycines* and *M. incognita* on soybean [30].

Aliphatic alchols may also be involved in the nematode suppression of velvet bean (*Mucuna aterrima*, Leguminosae). Fractionation from the velvet bean extract yielded 1-triacontanol (**14**) and triacontanyl tetracosanoate (**15**) as two main compounds to inhibit hatching of *M. incognita* at 1.0% [31,32].

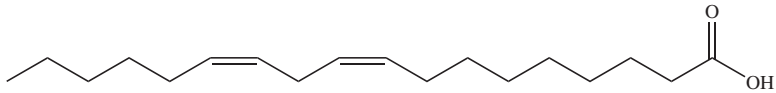
Plant-derived aliphatics could be easily synthesized. Some synthesized aliphatics for their nematicidal activity have also been investigated [33]. Structure–activity relationship showed that aliphatic alcohols, aldehydes and acids were more effective than hydrocarbons and acetates [33].



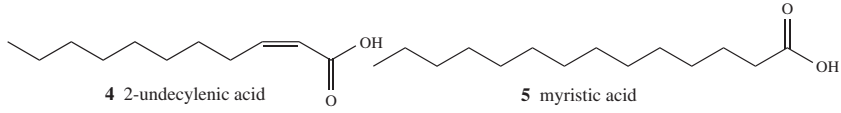
1 palmitic acid



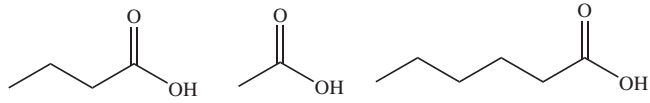
2 oleic acid



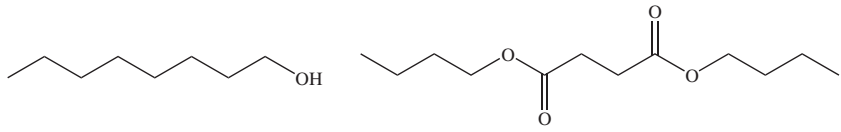
3 linoleic acid



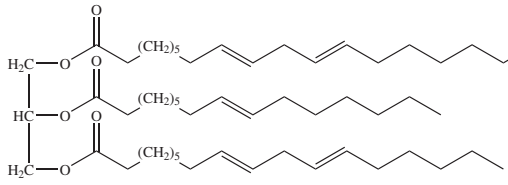
4 2-undecylenic acid **5** myristic acid



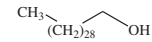
6 butyric acid **7** acetic acid **8** hexanoic acid



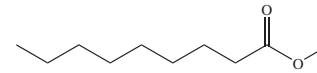
9 1-octanol **10** di-*n*-butyl succinate



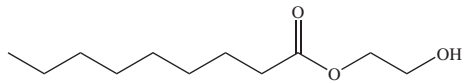
11 *sn*-glycerol 1-eicosa-9,12-dienoate-2-palmitoleate-3-linoleate



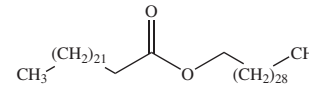
14 1-triacontanol



12 methyl pelargonate



13 ethylene glycol pelargonate



15 triacontanyl tetracosanoate

POLYACETYLENES

A broad spectrum of polyacetylenes with a variety of biological activities occur in the family Compositae, and many of them are nematicidal [21]. The first polyacetylene was identified from *Helenium* sp. (Compositae) as tridec-1-ene-3,5,7,9,11-pentayne (**16**), with nematicidal activity against *P. penetrans* and other nematodes [34].

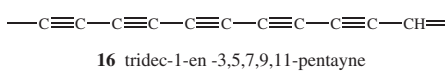
Two nematicidal polyacetylenes against *Bursaphelenchus lignicolus* were isolated from the roots of *Cirsium japonicum* (Compositae) and identified as tridec-1-ene-3,5,7,9,11-pentayne (**16**), and 9,10-epoxyheptadec-16-ene-4,6-diyn-8-ol (**17**) [35]. Other nematicidal polyacetylenes against *B. lignicolus* included 1-phenylhepta-1,3,5-triyne (**18**) and 2-phenyl-5-(1'-propynyl)thiophene (**19**) from *Coreopsis lanceolata* (Compositae), and *cis*-dehydromatricaria ester (**20**) from *Solidago altissima* (Compositae) [35].

Two nematicial polyacetylenes namely 2-*trans*-8-*cis*-matricaria ester (**21**) and 2-*cis*-8-*cis*-matricaria ester (**22**) against *Pratylenchus coffease* were isolated from the fresh roots of *Erigeron philadelphicus* (Compositae) [36].

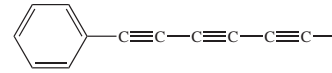
3-*cis*-11-*trans*-Trideca-1,3,11-triene-5,7,9-triyne (**23**) and its 3-*trans* isomer (**24**), obtained from the flowers of safflower (*Carthamus tinctorius*, Compositae) showed 30% mortality at 10μg/ml for compound **23**, and 85% mortality at 2μg/ml for compound **24** against the nematode *A. besseyi* after 24h [37,38].

Heptadeca-1,9-diene-4,6-diyne-3,8-diol (or named falcarindiol, **25**), from the fresh roots of *Angelica pubescens* (Umbelliferae), was nematicidal to *A. besseyi* [24].

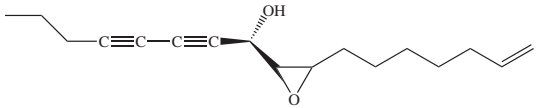
Some thiarubrines from compositous plants have a general toxicity and often require light for their maximum activity, similar to polythiophenes. One such compound, thiarubrine C (**26**), was isolated from the roots of the black-eyed Susan (*Rudbeckia hirta*, Compositae). This compound exhibited the LC_{50} values as 12.4μg/ml for *M. incognita*, and 23.5μg/ml for *P. penetrans*, in a motility and viability bioassay. It was found that the nematicidal activity of thiarubrine C (**26**) could be enhanced by light. Treatment of soil with 50μg/ml of thiarubrine C (**26**) decreased *M. incognita* infection of tomato seedlings by nearly 95% [39].



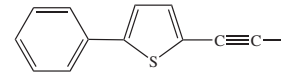
16 tridec-1-en -3,5,7,9,11-pentayne



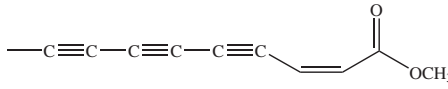
18 1-phenylhepta-1,3,5-triyne



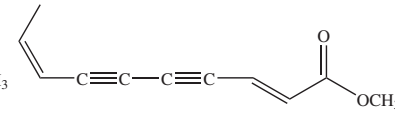
17 9,10-epoxyheptadec-16-en-4,6-diyn-8-ol



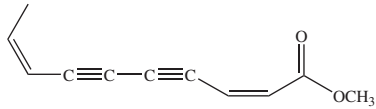
19 2-phenyl-5-(1'-propynyl)-thiophene



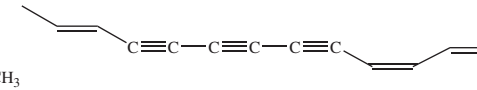
20 *cis*-dehydromatricaria ester



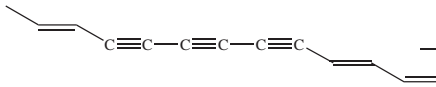
21 2-*trans*,8-*cis*-matricaria ester



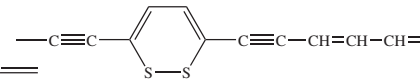
22 2-*cis*,8-*cis*-matricaria ester



23 3-*cis*,11-*trans*-trideca-1,3,11-triene-5,7,9-triyne



24 3-*trans*,11-*trans*-trideca-1,3,11-triene-5,7,9-triyne



26 thiarubrine C

25 heptadeca-1,9-diene-4,6-diyne-3,8-diol (falcarindiol)

POLYTHIOPHENES

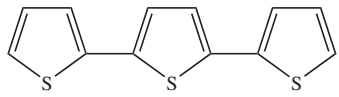
The best study case of a nematicidal principle from higher plants is probably that of polythiophenes (or called polythienyls) from marigolds (*Tagetes* spp., Compositae). Perhaps the resistance of marigolds to nematodes was first reported by Golf, who noted that French marigold (*Tagetes patula*) and African marigold (*Tagetes erecta*) were two of seven species devoid of root-knot nematodes (*Meloidogyne* spp.) infection during trials of 80 different ornamental annuals [4,22]. The growing plants, incorporated residues and root extracts of the *Tagetes* genus are generally effective at controlling a variety of nematodes in soil [40–42]. It has been well known that few of nematodes penetrating marigold roots reach maturity. Hundreds of reports have been appeared in the scientific literature of the frequently suppressive effects on nematode populations of marigolds, whether utilized as a cover crop, rotation crop, green manure, or source of nematode-antagonistic extracts. Marigolds showed multiple mechanisms of action against plant-parasitic nematodes including non-host or poor host effects, trap crop effect, production of antinematodal compounds, and stimulation of nematode natural enemies [4].

Uhlenbroek and Bijloo first clarified a thiophene compound as α-terthiophene (**27**) in *T. erecta* f.sp. *plena* [43]. α-Terthiophene (**27**) was nematicidal *in vitro* against potato cyst nematode *Globodera rostochiensis* at 0.1–0.2μg/ml, the wheat seed gall nematode (*Anguina tritici*) at 0.5μg/ml, and the stem and bulb nematode (*Ditylenchus dipsaci*) at 5μg/ml, respectively [43]. Subsequent works led to the identification of another polythiophene, 5-(1-buten-1-ynyl)-2,2'-bithiophene (**28**), which was active towards *Panagrellus redivirus* at 3.13μg/ml [44].

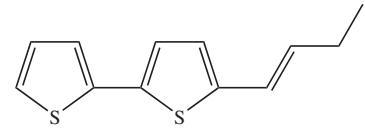
Of 175 tested compositous species, nearly 70 species effectively suppressed populations of the nematode *P. penetrans*. α-Terthiophene (**27**) was detected in *Eclipta prostrata*, *Flaveria repanda*, *Gaillardia pulchella*, *T. erecta*, *Tagetes petula*, *Tagetes tenuifolia*, *Berkheya adlamii*, *Berkheya macrocephala*, *Didelta carnosa*, and six *Echinops* species. Another nematocide, 5-(3-buten-1-ynyl)-2,2'-bithiophene (**29**), was detected in *Arnica sachalinensis*, *D. carnosa*, *E. prostrata*, *F. repanda*, three *Tagetes* species, two *Berkheya* species, and six *Echinops* species [45].

Three nematicidal thiophenes were isolated from *Tagetes* roots, and were identified as 5-(1-buten-1-ynyl)-2,2'-bithiophene (**28**), 5-(3-buten-1-ynyl)-2,2'-bithiophene (**29**), and 5-(4-acetoxy-1-butynyl)-2,2'-bithiophene (**30**) [46].

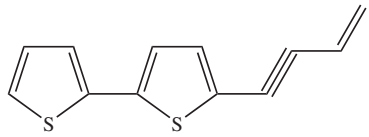
The roots of *Tagetes* species had the highest diversity and contents of thiophenes with 5-(3-buten-1-ynyl)-2,2'-bithiophene (abbreviated as BBT, **29**) as the main component followed by α-terthiophene (abbreviated as α-T, **27**) and 5-(4-hydroxybut-1-ynyl)-2,2'-bithiophene (abbreviated as BBTOH, **31**). By considering both concentrations and biomass yields of thiophene, *Tagetes minuta* and *Tagetes lucida* appeared to be the most promising *Tagetes* species, with high potential for use as biocidal crops [47].



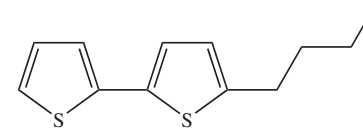
27 alpha-terthiophene



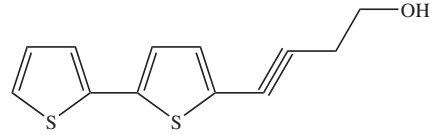
28 5-(1-buten-1-ynyl)-2,2'-bithiophene



29 5-(3-buten-1-ynyl)-2,2'-bithiophene (BBT)



30 5-butyl-2,2'-bithiophene

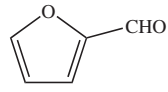


31 5-(4-hydroxybut-1-ynyl)-2,2'-bithiophene (BBTOH)

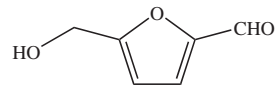
FURANS AND TETRAHYDROFURANS

The methanol extract of *M. azedarach* (Meliaceae) showed its nematicidal activity against *M. incognita*. Two isolated furans namely furfural (**32**) and 5-hydroxymethylfurfural (**33**) exhibited their nematicidal activities against the second-stage juveniles of the nematode *M. incognita*, and the nematical activity (LC_{50}, 8.5µg/ml) of furfural (**32**) was stronger than that (LC_{50}, 45.7µg/ml) of 5-hydroxymethylfurfural (**33**) [26]. Furfural (**32**) was also isolated from the sap of *Knema hookeriana* (Myristicaceae) and was active against *Bursaphelenchus xylophilus* in a cotton ball bioassay [48].

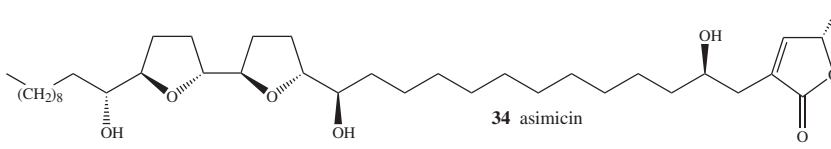
The annonaceous acetogenins have attracted considerable attention because of their antitumour and pesticidal activities [49,50]. These compounds were found only in the plant species of family Annonaceae, and were essentially derivatives of fatty acids. More than 350 acetogenins have been identified, and they contained either C-32 or C-34 chains. Some of them have been described as toxic to mosquito larvae, corn borers, aphids, beetles and free-living nematodes. A number of acetogenins have been tested for their antinematodal activities. Asimicin (**34**) was remarkably active (LC_{100}, 0.1µg/ml) towards *Caenorhabditis elegans*. Other acetogenins including annonacin (**35**), corossolin (**36**), murisolin (**37**), cherimolin (**38**), and otivarin (**39**) also exhibited inhibitory activity against *Molinema dessetae*. Antinematodal activity of these compounds was equivalent to that of ivermectin [49,50].



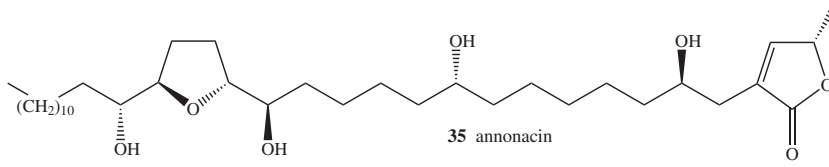
32 furfural



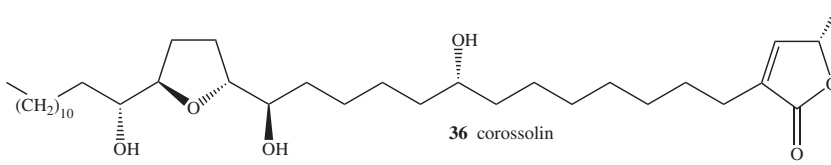
33 5-hydroxymethyl furfural



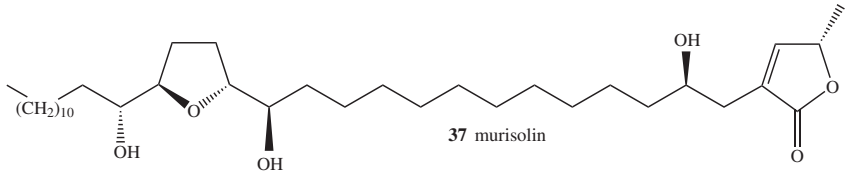
34 asimicin



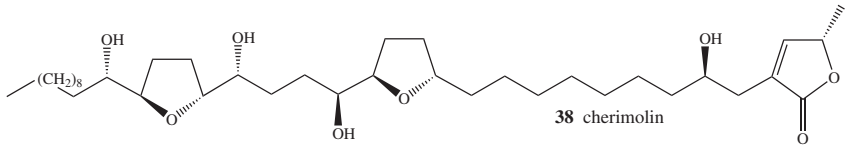
35 annonacin



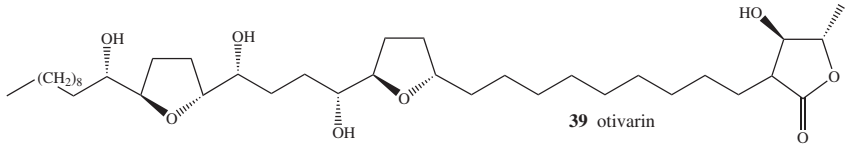
36 corossolin



37 murisolin



38 cherimolin



39 otivarin

TERPENOIDS

Monoterpenoids

A large number of phytochemicals called isoprenoids are formed by the condensation of five-carbon isoprene units. Among them, the simplest are 10-carbon compounds called mototerpenoids, which often are the major components of plant fragrants or essential oils. Monoterpenoids frequently possess activity against predators and pathogens.

The first monoterpene found to have anthelmintic activity was ascaridole (**40**), which was alone or as a component of plant extracts, has been used in the treatment of hookworm infection. This monoterpene was also a potent *in vitro* inhibitor of the development of the malarial parasite *Plasmodium falciparum* [21]. Twenty monoterpenoids were identified by GC–MS in the leaf essential oil of *Croton regelianus* (Euphorbiaceae). Ascaridole (**40**) accounted for 33.9% and was fractionated from the oil. The bioassay results showed that the essential oil of *C. regelianus* and ascaridole (**40**) were moderately active against the root-knot nematode *M. incognita* with LC_{50} values as 201.83μg/ml for the oil and 87.36μg/ml for ascaridole, but strongly effective with LC_{50} values as 24.22μg/ml (for the oil) and 9.60μg/ml (for ascaridole) against the larvae of *Aedes aegypti*, and 24.47μg/ml (for the oil) and 9.46μg/ml (for ascaridole) against the larvae of *Artemia* sp., respectively [51].

Thymol (**41**), a phenolic monoterpene existing in the essential oils of several plant families, exhibited strong antinematodal activity against plant-parasitic nematodes *Meloidogyne arenaria*, *H. glycines*, *Paratrichodorus minor* and free-living Dorylaimid nematodes. When it was added to soil at a concentration range of 25–250μg/g, population densities of the nematodes and disease incidence declined [52].

Other simple monoterpenes have been implicated as antinematodal agents, particularly when used as soil amendments: citronellal (**42**) [53]; citral (**43**), menthol (**44**), and α-terpineol (**45**) [48]; geraniol (**46**) [54]; and limonene (**47**) [55].

Menthol (**44**), geraniol (**46**), eugenol (**49**), linalool (**50**) and cineole (**51**), and a number of esters of menthol and geraniol, were toxic to the J_2 stage plant-parasitic nematodes *A. tritici*, *M. javanica* and *Tylenchulus semipenetrans* [56,57].

Four monoterpenoids, thymol (**41**) and carvacrol (**52**) from oregano (*Origanum* sp., Lamiaceae), *trans*-anethole (**53**) from fennel (*Foeniculum vulgare*, Umbelliferae), and L-carvone (**54**) from caraway (*Carum carvi*, Umbellifeae) were found to inhibit hatching of *M. javanica* at 125μg/l. When the compounds were incorporated into soil at 160mg/kg, all but thymol (**41**) completely inhibited root gall formation [58].

Geraniol (**46**), citronellol (**48**), and linalool (**50**) were obtained from the essential oil of *Pelargonium graveolens* (Geraniaceae) and induced mortality of *M. incognita* juveniles [59]. Seed treatment with citronellol (**48**) reduced *M. incognita* infection to tomato seedlings [60].

A synergistically acting combination of linalool (**50**) and methyl chavicol (or named as estragole, **55**) inhibited the motility of *Heterodera cajani* and *M. incognita*, whereas individual compounds were noneffective [61].

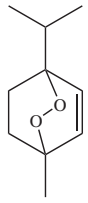
Palasonin (**56**), an antinematodal monoterpenoid derivative, was isolated from the seeds of *Butea frondosa* (Leguminosae). The anthelmintic action of this compound on *Ascaridia galii* possibly involved inhibition of glucose uptake and/or of the motor activity of the parasite [62].

Nematicidal activities of 22 monoterpenoids were evaluated both *in vitro* and in pot experiments. Twenty of them significantly inhibited hatching, and 11 inhibited J_2 mobility of the root-knot nematode (*M. incognita*) at 250mg/l. Structure–activity relationships revealed that the compounds with hydroxyl and carbonyl groups exhibited higher nematicidal activity than other terpenoids. Citral (**43**), α-terpineol (**45**), geraniol (**46**), L-borneol (**57**), and carveol (**58**) showed the highest nematicidal activity among the monoterpenoids tested in vitro. These compounds drastically reduced eggs hatching and J_2 viability, and diminished root gall formation of tomato plants in pot experiments [63].

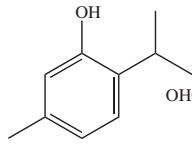
Eight monoterpenoids, estragole (**55**), L-borneol (**57**), α-pinene (**59**), β-pinene (**60**), δ-3-carene (**61**), L-camphor (**62**), α-terpinolene (**63**), and benzaldehyde (**64**) along with two sesquiterpenoids namely caryophyllene oxide (**65**) and γ-eudesmol (**66**) were isolated from seven plant species *Eucalyptus meliodora* (Myrtaceae), *Juglans regia* (Juglandaceae), *Laurus nobilis* (Lauraceae), *Pistacia terebinthus* (Anacardiaceae), *F. vulgare* (Umbelliferae), *Pimpinella anisum* (Umbelliferae) and *Achillea millefolium* (Compositae) indigenous to Greece. They were screened for their antinematodal activity on *M. incognita* [64]. Of these compounds, benzaldehyde (**64**) (LC_{50}, 9μg/ml) was the most toxic, followed by γ-eudesmol (**66**) (LC_{50}, 50μg/ml) and estragole (**55**) (LC_{50}, 180μg/ml). The synergistic, additive and antagonistic effects of each two terpenes selected from the following nine compounds, thymol (**41**), geraniol (**46**),

eugenol (**49**), carvacrol (**52**), *trans*-anethole (**53**), L-carvone (**54**), estragole (**55**), γ-eudesmol (**66**), and pulegone (**67**) were also investigated. The most potent terpene pairs which existed synergistic effects were geraniol (**46**)/*trans*-anethole (**53**), eugenol (**49**)/*trans*-anethole (**53**), eugenol (**49**)/carvacrol (**52**), and geraniol (**46**)/carvacrol (**52**), in their decreasing order [64].

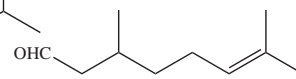
The nematicidal activity of thymol (**41**) and carvacrol (**52**) on *C. elegans* might be mediated through TyrR as the two compounds could trigger the signalling cascade downstream from the receptor in cells expressing wild-type but not a mutant SER-2. The TyrR-expressing cell system may prove to be a good screening platform for developing new nematicidal compounds [65].



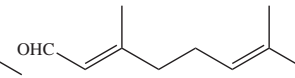
40 ascaridole



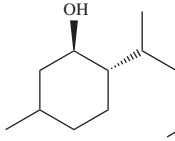
41 thymol



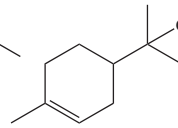
42 citronellal



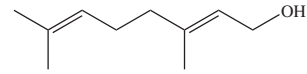
43 citral



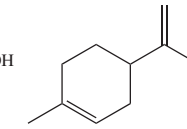
44 menthol



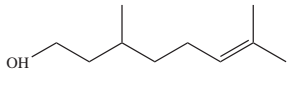
45 alpha-terpineol



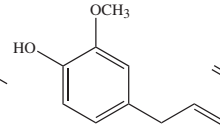
46 geraniol



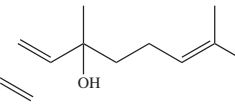
47 limonene



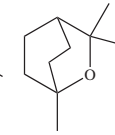
48 citronellol



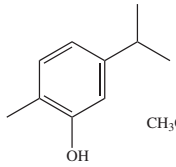
49 eugenol



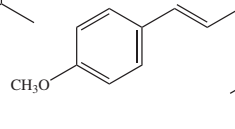
50 linalool



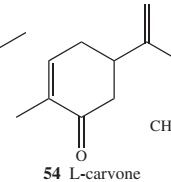
51 cineole



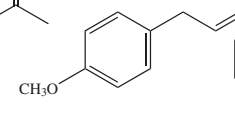
52 carvacrol



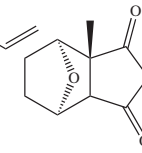
53 *trans*-anethole



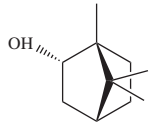
54 L-carvone



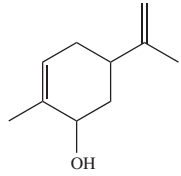
55 methyl chavicol
= estragole



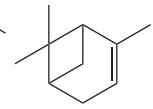
56 palasonin



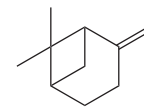
57 L-borneol



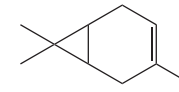
58 carveol



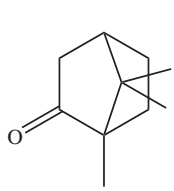
59 alpha-pinene



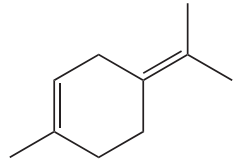
60 beta-pinene



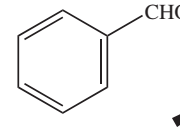
61 delta-3-carene



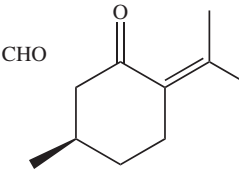
62 L-camphor



63 alpha-terpinolene



64 benzaldehyde



67 pulegone

Sesquiterpenoids

Sesquiterpenoids are C_{15} compounds formed by the condensation of three isoprene units. The first sesquiterpenoids discovered to be nematotoxic were the terpenoid aldehydes hemigossypol (**68**), 6-methoxyhemigossypol (**69**) and the C_{30} dimers gossypol (**70**) and 6-methoxygossypol (**71**) from cotton (*Gossypium hirsutum*, Malvaceae). Postinfection production of these compounds in a resistant cotton variety was associated with resistance to *M. incognita* [66]. A crude terpenoid aldehyde extract from cotton inhibited movement of *M. incognita* juveniles at 50µg/ml, and gossypol (**70**) showed the equivalent effect at 125µg/ml [67]. In contrast, a more comprehensive analysis of the roots of six cotton cultivars failed to reveal the correlation between nematode resistance and root (or leaf) terpenoid aldehyde content or composition [68].

8α-Hydroxyelemol (**72**) from the berries of *Juniperus* sp. (Cupressaceae) showed moderate nematicidal activity at 80µg/ml against *C. elegans* [69].

Alantolactone (**73**) from *Inula helenium* (Compositae) with a mammalian anthelmintic activity showed nematicidal activity at 1100µg/ml *in vitro* against *M. incognita*. Maximum toxicity was associated with an α-methylene-γ-lactone moiety; modification of this group reduced activity [70].

Genetic analysis indicated that the production of the sesquiterpenoid phytoalexin solavetivone (**74**) was genetically linked to resistance of potato (*Solanum tuberosum*, Solanaceae) against *G. rostochiensis* [71].

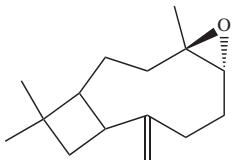
Another sesquiterpenoid phytoalexin namely rishitin (**75**) was identified in potato tuber discs infected with the potato rot nematode *Ditylenchus destructor* and *D. dipsaci*. The LC_{50} of rishitin (**75**) in a motility assay against *D. dipsaci* was 100µg/ml [72]. A number of *in vitro* and small-pot studies also demonstrated antinematodal effect of rishitin (**75**) on *Xiphinema diversicaudatum* [73].

α-Humulene (**76**), which repelled *B. xylophilus* and occurred in a heartwood extract of *Pinus massoniana* (Pinaceae), was one of the rare sesquiterpenes repelling plant-parasitic nematodes [74].

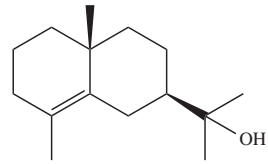
Santonin (**77**), a potent anthelmintic from compositous plants, has been used to treat ascaridiasis and oxyuriasis, but several cases of fatal poisoning by santonin (**77**) have been reported. Small doses may have effects on vision, and cause headache, nausea and vomiting. Higher doses may cause epileptiform convulsions. Santonin (**77**) acted on the ganglion cells of the worm to induce paralysis so that the parasite could be eliminated *via* the feces [75].

Artemisin (**78**) from *Artemisia annua* (Compositae) is another sesquiterpene lactone reported to have anthelmintic properties [76].

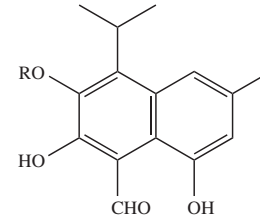
The leaf ethanol extract of *Magnolia grandiflora* (Magnoliaceae) exhibited strong nematicidal activity against *B. xylophilus* and *Panagrellus redivivus*, causing 73% and 100% mortality respectively within 48h at 5mg/ml. A nematicidal sesquiterpene namely 4,5-epoxy-1(10)*E*,11(13)-germacradien-12,6-olide (**79**) was obtained, and its median lethal concentrations (LC_{50}) against *B. xylophilus* and *P. redivivus* were 71µg/ml and 46µg/ml, respectively, at 48h [77].



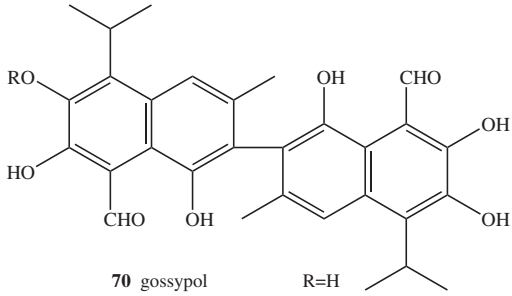
65 caryophyllene oxide



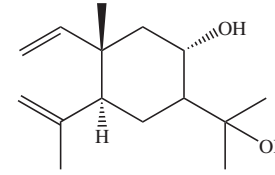
66 gamma-eudesmol



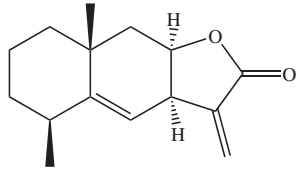
68 hemigossypol R=H
69 6-methoxyhemigossypol R=CH_3



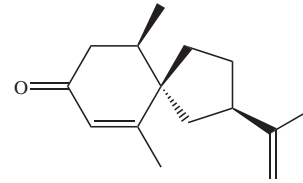
70 gossypol R=H
71 6-methoxygossipol R=CH_3



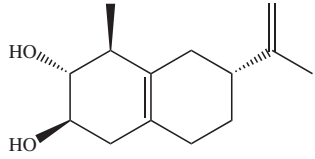
72 8-alpha-hydroxyelemol



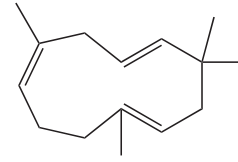
73 alantolactone



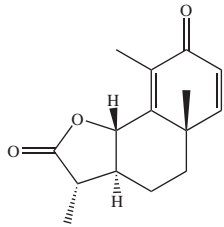
74 solavetivone



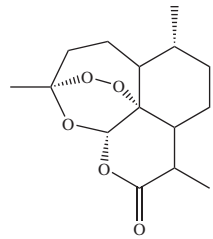
75 rishtin



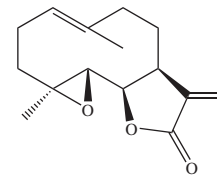
76 alpha-humulene



77 santonin



78 artemisinin

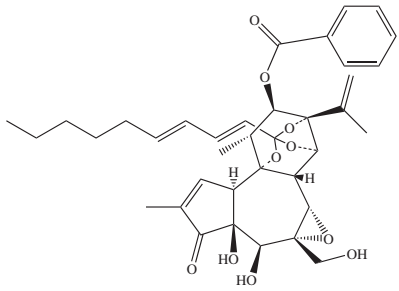


79 4,5-epoxy-1(10)E,11(13)-germacradien-12,6-olide

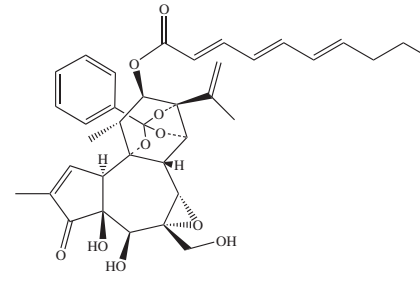
Diterpenoids

Some diterpenoids from plants were reported to have their antinematodal activity. Two diterpenoids, odoracin (**80**) and odoratrin (**81**) were isolated from the nematocidal extract of *Daphne odora* (Thymelaeaceae) roots. Odoracin (**80**) was nematotoxic to *A. besseyi* at 5.0μg/ml and consisted of a diterpenoid skeleton esterified benzoic acid as well as a fatty acid. Odoratrin (**81**) possessed an obvious antinematodal activity at 5.0μg/ml [24,78].

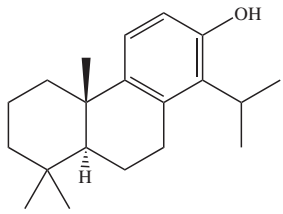
Totarol (or named 14-isopropyl podocarpa-8,11,13-trien-13-ol, **82**) and ferruginol (or named 13-isopropyl podocarpa-8,11,13-trien-12-ol, **83**) were isolated from the berries of three cupressaceous plants *Juniperus procera*, *J. excelsa*, and *J. phoenicea*. Totarol (**82**) showed strong nematicidal activity at 80μg/ml against *C. elegans*, and ferruginol (**83**) exhibited moderate nematicidal activity at 80μg/ml [69]. Three diterpenoids: 20-*O*-acetyl-[3-*O*-(2'E,4'Z)-decadienoyl]-ingenol(**84**),20-*O*-acetyl-[5-*O*-(2'E,4'Z)-decadienoyl]-ingenol (**85**) and 3-*O*-(2'E,4'Z)-decadienoylingenol (**86**), were isolated from the roots of *Euphorbia kansui* (Euphorbiaceae) under the bioassay-guided method. They showed the similar antinematodal activity against *B. xylophilus*, at a minimum lethal dose (MLD) as 5g/cotton ball [79]. In addition, some diterpenoids in plant essential oils may contribute to the antinematodal activity [18].



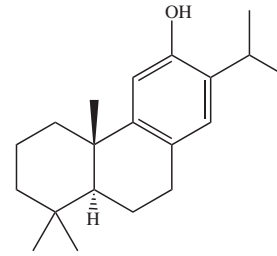
80 odoracin



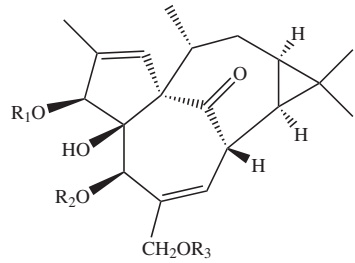
81 odoratrin



82 totarol



83 ferruginol



84 20-*O*-acetyl-[3-O-(2'E,4'Z)-decadienoyl]-ingenol R_1=CO-(CH=CH)$_2$-(CH$_2$)$_4$-CH$_3$; R_2=H; R_3=COCH$_3$
85 20-*O*-acetyl-[5-O-(2'E,4'Z)-decadienoyl]-ingenol R_1=H; R_2=CO-CH=CH=CH-(CH$_2$)$_4$-CH$_3$; R_3=COCH$_3$
86 3-*O*-(2'E,4'Z)- decadienoylingenol R_1=CO-CH=CH-CH=CH-(CH$_2$)$_4$-CH$_3$; R2=H; R_3=H

Triterpenoids

Triterpenoids are the compounds containing six isoprene units. Their glycosides are called triterpenoid saponins. Three triterpenoids: azadirachtins A (**87**), B (**88**) and H (**89**) isolated from *Azadirachta indica* (Meliaceae) showed LC_{50} values of 460.7µg/ml, 125.8µg/ml and 229.4µg/ml, respectively, against the second-stage juveniles of root-knot nematode, *Meloidgyne incognita* [80], and LC_{50} values of 119.1µg/ml, 96.6µg/ml and 141.2µg/ml, respectively, against *Rotylenchulus reniformis*. At 200µg/ml, all the three azadirachtins caused 50–65% mortality of *C. elegans* [81].

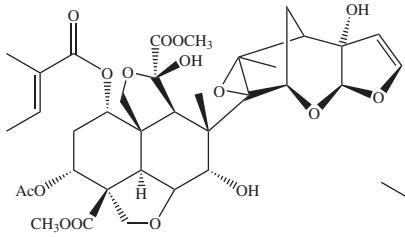
The triterpenoid camarinic acid (**90**) from *Lantana camara* (Verbenaceae) killed *M. incognita* juveniles at 1.0% [82]. Twelve triterpenoids were further isolated from the aerial parts of *L. camara* (Verbenaceae). Of these compounds, pomolic acid (**91**), lantanolic acid (**92**), and lantoic acid (**93**) showed 100% mortality on root-knot nematode *M. incognita* at 1mg/ml after 24h, while camarin (**94**), lantacin (**95**), camarinin (**96**), and ursolic acid (**97**) exhibited 100% mortality at 1mg/ml after 48h. The positive control furandan showed 100% mortality on the nematode at 1mg/ml after 24h [83].

In addition, lantanilic acid (**98**), oleanolic acid (**99**) and camaric acid (**100**) with nematicidal activity were isolated through bioassay-guided fractionation from the methanolic extract of the aerial parts of *L. camara*. They exhibited 98%,

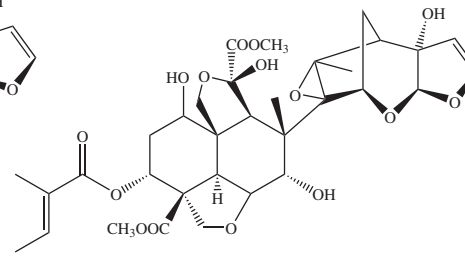
95% and 70% mortality, respectively, against root-knot nematode *M. incognita* at 0.5% [84].

Bioactivity-guided fractionation from the leaves of *Ocimum gratissimum* (Lamiaceae), a plant used as an anthelimintic in Nigeria, led to the isolation of oleanolic acid (**99**) which killed *C. elegans* at 1.0μg/ml [85].

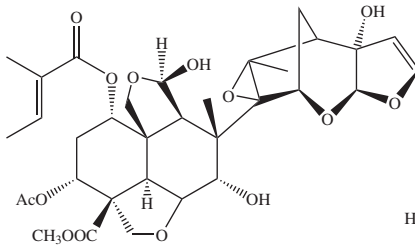
The pure saponin mixtures and eight sapogenins namely oleanolic acid (**99**), 2β-hydroxy oleanolic acid (**101**), bayogenin (**102**), hederagenin (**103**), 2β,3β-dihydroxy-23-oxo-olean-12-en-28-oic acid (**104**), medicagenic acid (**105**), zanhic acid (**106**), and soyasapogenol B (**107**) from three *Medicago* species (*M. arborea*, *M. arabica*, and *M. sativa*) in Leguminosae family were investigated for their activity against the plant-parasitic nematode *Xiphinema index*. Among the sapogenins, hederagenin (**103**) with 57% mortality at 15.6μg/ml after 24h of treatment was found to be the most active compound. Medicagenic acid (**105**) with 52% mortality at 62.5μg/ml after 48h of treatment appeared to be slightly more active than bayogenin (**102**) [86]. Both saponins and sapogenins from *Medicago* spp. might be good candidates for natural nematicide formulations, especially against the virus-vector nematode *X. index*, the root-knot nematode *M. incognita* and the potato cyst parasite *G. rostochiensis* [87]. Large biomass produced by *Medicago* species could make the industrial extraction of saponins economically viable. Soil amendments with *M. sativa* pelleted meal has been demonstrated to be strongly suppressive on root-knot and cyst nematode species even though in the presence of heavy infestation conditions and, therefore, could be hypothesized as a valuable option for an environmental safe nematode management [87,88].



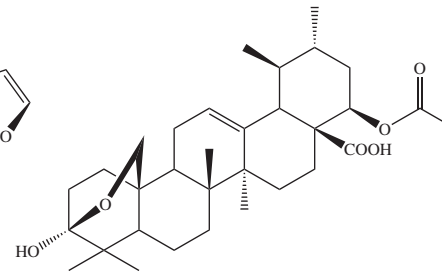
87 azadirachtin A



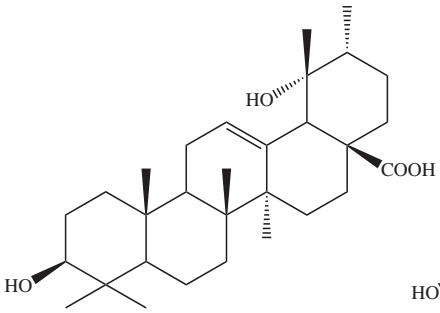
88 azadirachtin B



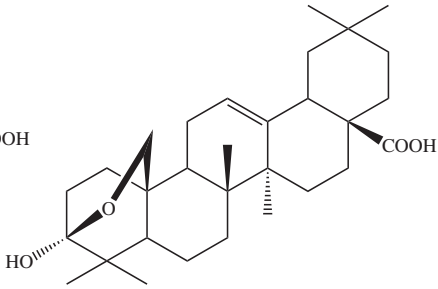
89 azadirachtin H



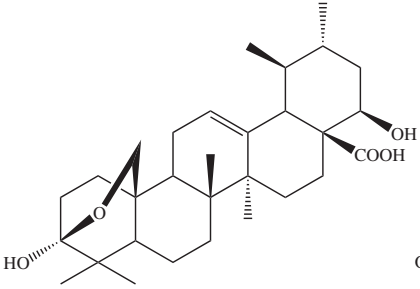
90 camarinic acid



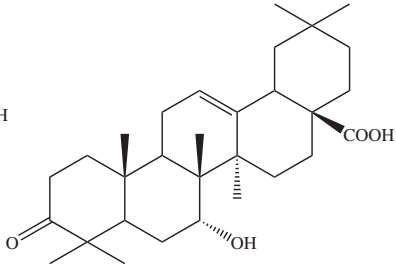
91 pomolic acid



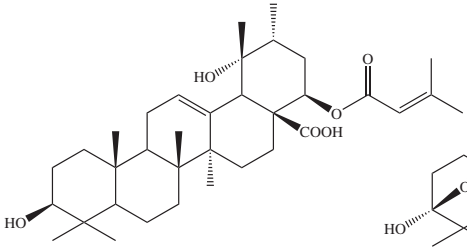
92 lantanolic acid



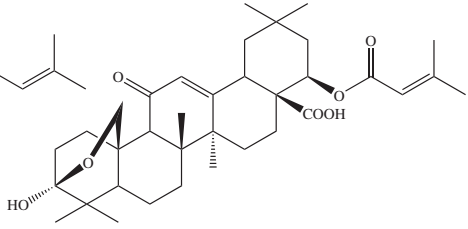
93 lantoic acid



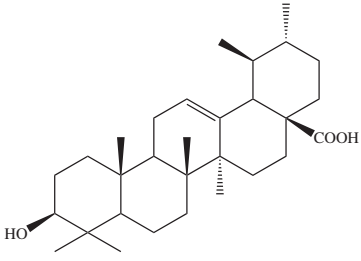
94 camarin



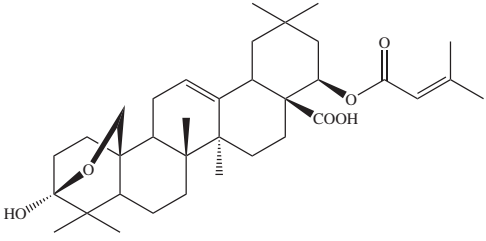
95 lantacin



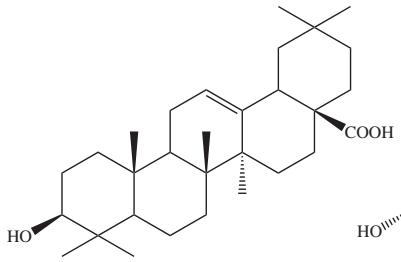
96 camarinin



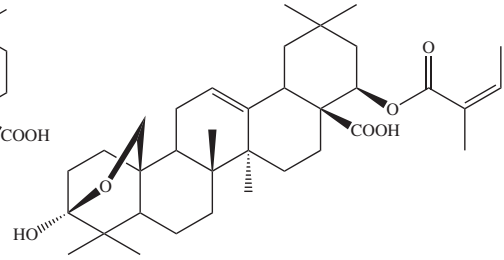
97 ursolic acid



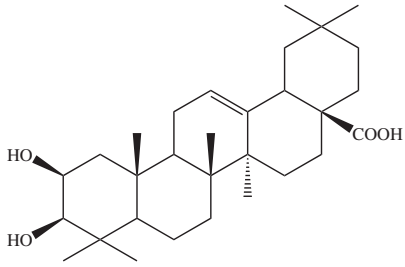
98 lantanilic acid



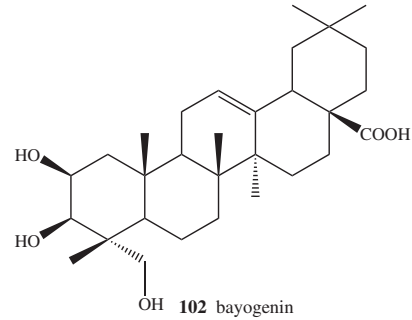
99 oleanolic acid



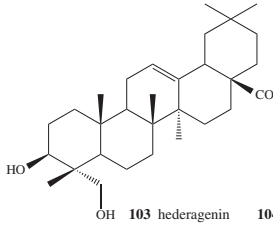
100 camaric acid



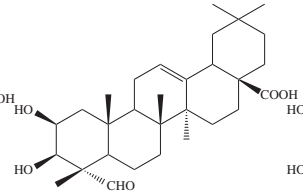
101 2β-hydroxy oleanolic acid



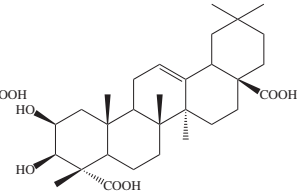
102 bayogenin



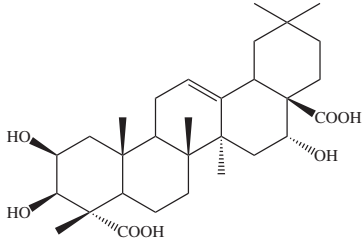
103 hederagenin



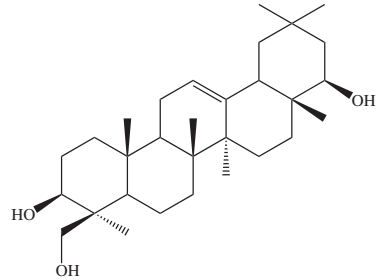
104 2β,3β-dihydroxy-23-oxo-olean-12-en-28-oic acid



105 medicagenic acid



106 zanhic acid



107 soyasapogenol B

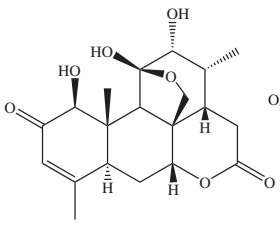
QUASSINOIDS

Quassinoids are all-chair cyclic and highly oxygenated products of squalene. They biogenetically can be regarded as the degraded triterpenoids, which were isolated exclusively as bitter principles from the plants of Simaroubaceae family

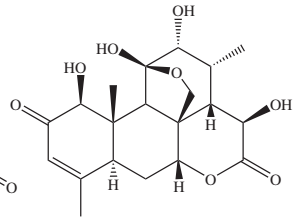
[89–91]. The quassinoids have a variety of biological activities including antifeedant, inseticidal, herbicidal, antiparasitic, antimalarial, and anticancer properties, and can be divided into distinct groups according to their basic skeletons C_{18}, C_{19}, C_{20}, C_{22} and C_{25} [89–91].

Three quassinoids namely chaparrinone (**108**), glaucarubolone (**109**), and klaineanone (**110**) were isolated from the seeds of *Hannoa undulata* (Simarubaceae). They inhibited penetration of tomato roots by *M. javanica* juveniles at 1–5μg/ml [92,93].

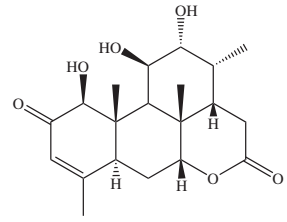
Watanabe *et al.* selected 38 quassinoids isolated from simaroubaceous species to evaluate their nematocidal activity by using a free-living nematode of the Diplogastridae family to develop the lead parasiticides samaderines B (**111**) and E (**112**) from the stem bark of *Quassia indica* (Simaroubaceae). Both the compounds (**111** and **112**) displayed the most potent nematocidal activity with a minimum lethal concentration (MLC) of 2.0×10^{-5}mol/l. The nematocidal activities of samaderines B and E were 15-fold greater than that of albendazole (3.0×10^{-4}mol/l), 10-fold greater than that of thiabendazole (2.0×10^{-4}mol/l) and 7.5-fold greater than that of avermectin (1.5×10^{-4}mol/l) [94]. Other nematocidal quassinoids with strong activity included cedronin (**113**), brucein B (**114**), brucein D (**115**), and brusatol (**116**). In addition, the cytotoxicity of brucein D (**115**) from *Brucea mollis* var. *tonkinensis* was comparatively low though its antinematodal activity was not very strong [94]. Kuriyama *et al.* suggested that samaderine B (**111**) shared a common binding site with the γ-aminobutyric acid receptor (GABAR) antagonist 4-cyclohexyl-3-isopropyl-BPT in Diplogastridae [95].



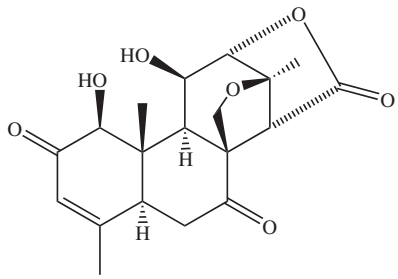
108 chaparrinone



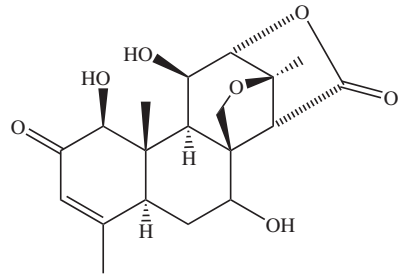
109 glaucarubolone



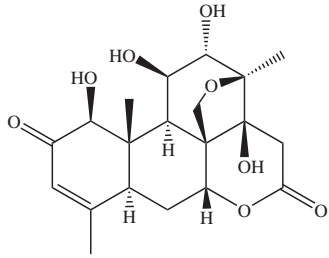
110 klaineanone



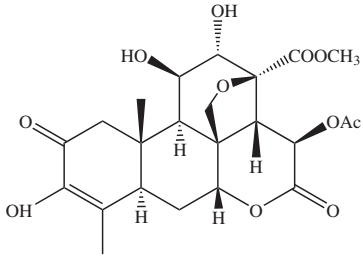
111 samaderine B



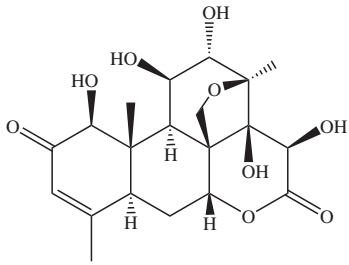
113 cedronin



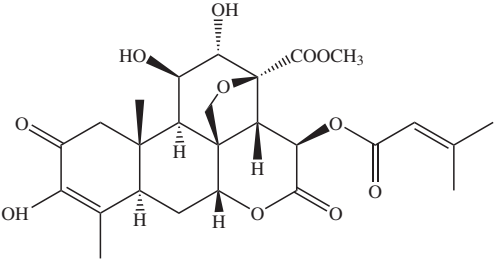
112 samaderine E



114 brucein B



115 brucein D

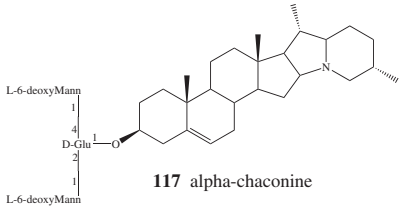


116 brusatol

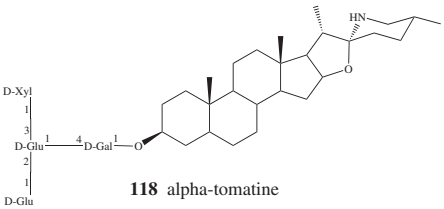
STEROIDS

Plant steroids arise through the intermediacy of cycloartenol and 2,3-oxidosqualene: cycloartenol cyclase (EC 5.4.99.8). They have been reported to possess multi-biological activities such as growth-regulating activity and developmental modification properties in plants. Two solanaceous steroidal glycoalkaloids, α-chaconine (**117**) and α-tomatine (**118**), were toxic to *P. redivivus* [96,97]. A mixture of two glycosides, protodioscin (**119**) and deltoside (**120**) from *Dioscorea deltoidea* (Dioscoreaceae) inhibited *M. incognita* motility at 5mg/ml but decreased nematode infection of tomato roots at a very low concentration *via* a host-mediated effect [98].

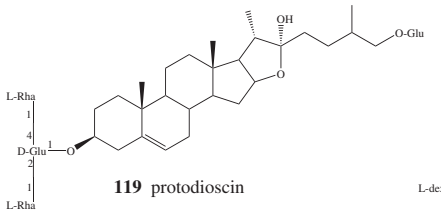
Three nematicidal cardenolides: 3β-*O*-(β-D-diginosyl)-14,15α-dihydroxy-5α-card-20(22)-enolide (**121**), uzarigenin (**122**) and cardenolide N-1 (**123**) were obtained from the ethyl acetate extract of *Nerium indicum* (Apocynaceae) by bioassay-guided fractionation. The median lethal concentrations (LC_{50}) of the compounds against the nematodes *B. xylophilus*, *P. redivivus*, and *C. elegans* after a period of 72h were 103.3, 49.0 and 45.4μg/l; 257.0, 62.7 and 177.8μg/l; and 242.9, 29.1 and 41.7μg/l, respectively [99].



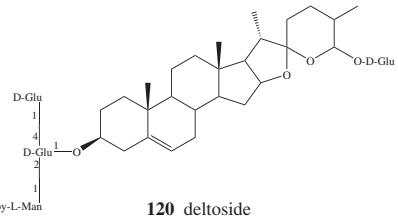
117 alpha-chaconine



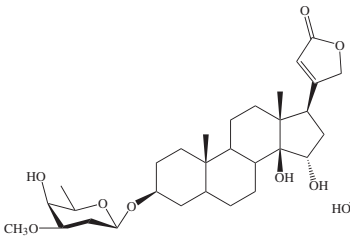
118 alpha-tomatine



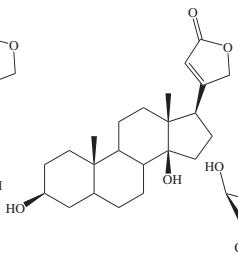
119 protodioscin



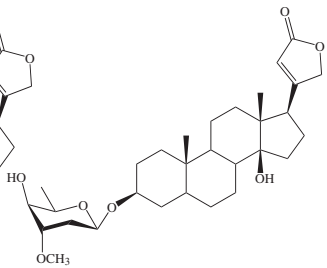
120 deltoside



121 3β-O-(β-D-diginosyl)-14,15 -dihydroxy-5 - card-20(22)-enolide



122 uzarigenin



123 cardenolide N-1

PHENOLS AND PHENOLIC ACIDS

Most plant phenolics arise from the shikimic and acetate pathways, which begin with the action of phenylalanine ammonia lyase upon phenylalanine or tyrosine [100]. As phenolics and lignification have been associated with plant resistance to a variety of pests and pathogens, investigations have sometimes revealed a correlation of elevated levels of phenolics with resistance or response of plants to nematode infection [101]. Many simple phenols are volatile, and exist in plant essential oils with antinematodal activity.

Pyrocatechol (**124**) isolated from *Eragrostis curvula* (Gramineae) was highly toxic to root-knot nematode juveniles. Its antinematodal effect was still detectable at 1:108 dilution [102]. Salicylic acid (**125**) and 4-hydroxybenzoic acid (**126**) showed nematocidal activity towards *C. elegans* and *M. incognita* and were effective in drench application [103].

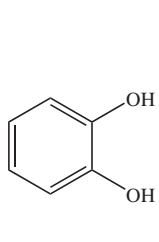
Both methyl 4-hydroxybenzoate (**127**) and methyl 4-hydroxycinnamate (**128**) isolated from *A. grayi* showed inhibitory activity on *M. incognita* [27].

Immersion of tomato roots in solutions of five phenolics namely pyrocatechol (**124**), hydroquinone (**129**), phloroglucinol (**130**), pyrogallol (**131**), and orcinol (**132**) inhibited the infection by *R. reniformis* [104].

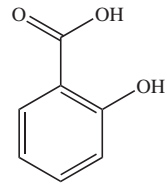
Seven phenolic compounds including pyrogallol (**131**), ethyl gallate (**133**), 3,5-dihydroxybenzoic acid (**134**), 3,4-dihydroxybenzoic acid (**135**), *trans*-cinnamic acid (**136**), frulic acid (**137**) and caffeic acid (**138**) were highly toxic to the larvae of *M. incognita* [105]. Both syringic acid (**139**) and vanillic acid (**140**) from the waste water of *Olea europaea* (Oleaceae) oil mills showed antinematodal activity on *M. javanica* at 15µg/ml [106].

Two phenolic acids, 3,5-dihydroxybenzoic acid (**134**) and gallic acid (**141**), were isolated from the aerial parts of *Rubus niveus* (Rosaceae) with their nematicidal activity against freshly hatched secondary stage juveniles of *M. incognita* [107]. Another two nematicial phenolic compounds namely vanillic acid (or called 3-methoxy-4-hydroxy benzoic acid, **140**) and methyl benzoate (**142**) against nematode *M. incognita* were isolated from the aerial parts of *Buddleja crispa* (Loganiaceae) [108].

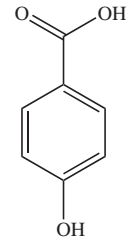
Furthermore, the condensed tannins extracted from *Lotus pedunculatus* (Leguminosae), *Lotus corniculatus* (Leguminosae), *Dorycnium pentaphyllum* (Leguminosae), *Dorycnium rectum* (Leguminosae) and *Rumex obtusifolius* (Polygonaceae) were able to disrupt the life cycle of the nematode *Teladorsagia circumcincta* by affecting egg hatching and larval development [109].



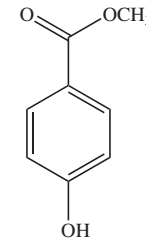
124 pyrocatechol



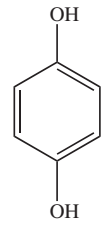
125 salicylic acid



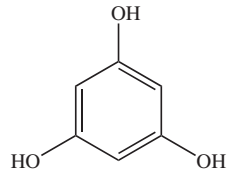
126 4-hydroxybenzoic acid



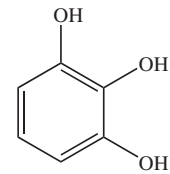
127 methyl 4-hydroxybenzoate



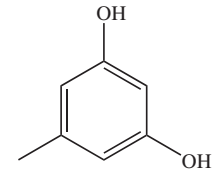
129 hydroquinone



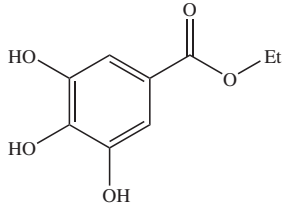
130 phloroglucinol



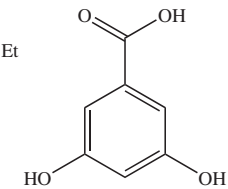
131 pyrogallol



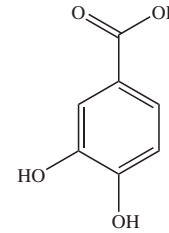
132 orcinol



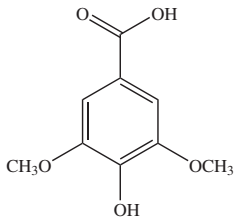
133 ethyl gallate



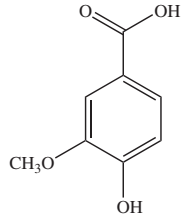
134 3,5-dihydroxybenzoic acid



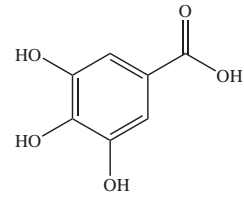
135 3,4-dihydroxybenzoic acid



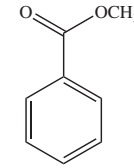
139 syringic acid



140 vanillic acid



141 gallic acid



142 methylbenzoate

PHENYLPROPANOIDS AND LIGNANS

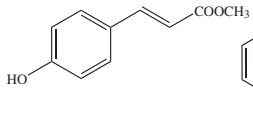
Five propenylphenols as chavicol (**143**), chavibetol (**144**), chavibetol acetate (**145**), allylpyrocatechol (**146**), and allypyrocatechol diacetate (**147**) from the chloroform extract of leaves of *Piper betle* (Piperaceae) killed *C. elegans* at 200μg/ml. The high levels of these compounds in the leaves could constitute an efficient nematocidal effect [110]. Using microspectrofluorometry and image analysis, it was found that chavicol (**143**) and allypyrocatechol (**146**) caused a significant increase in levels of Ca^{2+} in the intestinal tract and destruction of the cell membrane, allowing leakage of cytosol from the intestinal tract into the pseudocoelomic cavity. The compounds induced a higher level of Ca^{2+}, but for a shorter period, than DOPA [111].

Bioassay-directed fractionation with *C. elegans* led to the identification of chavicol (**143**) and demethyleugenol (or called allypyrocatechol, **146**) from wounded leaves of *Viburnum furcatum* (Caprifoliaceae), and 4-vinylphenol (**148**) from wounded leaves of three rosaceous species *Sorbaria sorbifolia*, *Spiraea salicifolia*, and *Malus baccata*. The minimum effective concentrations of these compounds ranged from 300 to 600μg/ml towards *C. elegans* [112].

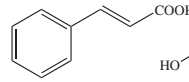
Both caffeic acid (**138**) and *o*-coumaric acid (**149**) from the waste water of *O. europaea* (Oleaceae) oil mills showed antinematodal activity on *M. javanica* at 15μg/ml [106].

Acorus gramineus (Araceae) has been used as an insecticide for a long time [113]. The hexane extracts of the plant showed *in vitro* nematocidal activity. The activity was shown to be due to α-asarone (**150**) and β-asarone (**151**) [114], which were proved to have mammalian toxicity and carcinogenicity, but not suitable for use as pesticide [115].

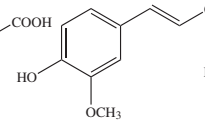
Two antinematodal propenylphenols magnolol (**152**) and honokiol (**153**) were isolated from the ethyl acetate extract of the branches of *Magnolia tripetala* (Magnoliaceae). Their median lethal concentration (LC_{50}) values were 149.3μg/ml and 63.7μg/ml, respectively, against *B. xylophilus*; 74.5μg/ml and 75.9μg/ml, respectively, against *P. redivivus*; 64.7μg/ml and 57.8μg/ml, respectively, against *C. elegans* [116].



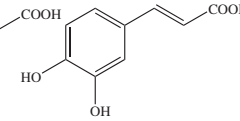
128 methyl 4-hydroxy cinnamate



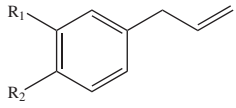
136 *trans*-cinnamic acid



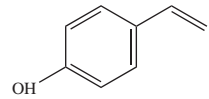
137 ferulic acid



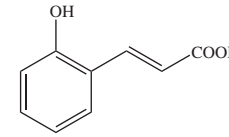
138 caffeic acid



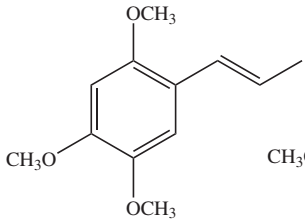
143 chavicol R_1=H, R_2=OH
144 chavibetol R_1=OH, R_2=OCH_3
145 chavibetol acetate R_1=OAc, R_2=OCH_3
146 allylpyrocatechol R_1=OH, R_2=OH
147 allylpyrocatechol diacetate R_1=OAc, R_2=OAc



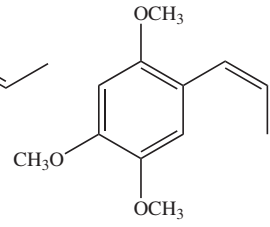
148 4-vinylphenol



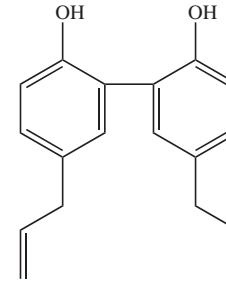
149 *o*-coumaric acid



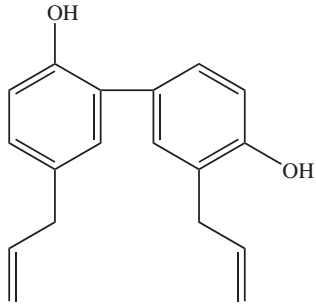
150 alpha-asarone



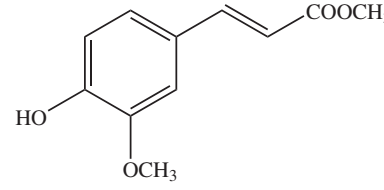
151 beta-asarone



152 magnolol



153 honokiol



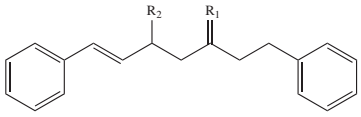
172 methyl ferulate

Lignans are phenylpropanoid dimmers. Five nematicidal diphenylheptanoids, 1,7-diphenyl-3-acetoxy-6(*E*)-heptene (**154**), 1,7-diphenyl-6(*E*)-hepten-3-one (**155**), 1,7-diphenyl-6(*E*)-hepten-3-ol (**156**), 1,7-diphenyl-4(*E*),6(*E*)-heptadien-3-ol (**157**), and 1,7-diphenyl-6(*E*)-hepten-3-one-5-ol (**158**), with LC_{95} values as low as 0.7μg/ml in motility assays against *C. elegans* were isolated from the roots of turmeric (*Curcuma comosa*, Zingiberaceae) [117].

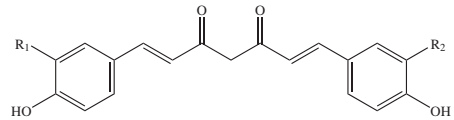
Four curcuminoids namely curcumin (**189**), demethoxycurcumin (**160**), bisdemethoxycurcumin (**161**) and cyclocurcumin (**162**) were isolated from the nematocidally active extract of *Curcuma longa* (Zingiberaceae). When tested individually in an assay using *Toxocara canis*, the compounds were ineffective. However, a mixture of the compounds showed activity (0.1µg/ml) suggesting a synergistic action between them [118].

The methanol extract of *Myristica malabarica* (Myristicaceae) fruit rinds showed nematicidal activity with mortality of 100% at 1000µg/ml against *B. xylophilus*. Three nematicidal substances: malabaricones A (**163**), B (**164**) and C (**165**) were bioassay-guided and fractioned. Both malabaricones B (**164**) and C (**165**) showed stronger activity than malabaricone A (**163**). At the same time, a significant synergistic interaction between the three compounds on the pine wood nematode *B. xylophilus* was observed. When the combination of them at a ratio of 1:1:1 was applied, its LC_{50} value was 11.8µg/ml [119].

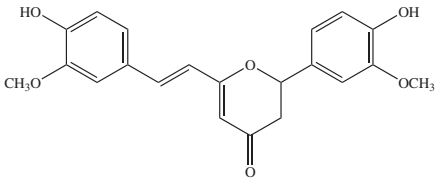
Dihydroguaiaretic acid (**166**) fractionated from the stem barks of *Pycnanthus angolensis* (Myristaceae) showed its antinematodal activity with the LC_{50} value as 10µg/ml on *C. elegans* [120].



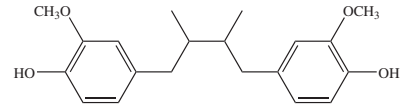
154 1,7-diphenyl-3-acetoxy-6(*E*)-heptene R_1=H, $OCOCH_3$; R_2=H
155 1,7-diphenyl-6(E)-hepten-3-one R_1=O; R_2=H
156 1,7-diphenyl-6(E)-hepten-3-ol R_1=H, OH; R_2=H
157 1,7-diphenyl-4(E),6(E)-heptadien-3-ol R_1=H, OH; R_2=H; delta^{4,5}
158 1,7-diphenyl-6(E)-hepten-3-one-5-ol R_1=O; R_2=OH



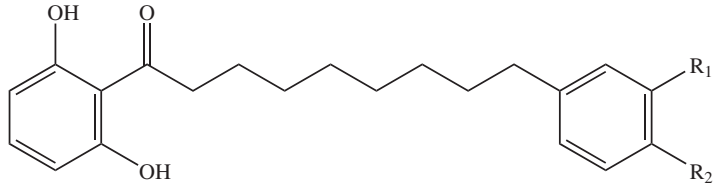
159 curcumin R_1=R_2=OCH_3
160 demethoxycurcumin R_1=OCH_3; R_2=H
161 bisdemethoxycurcumin R_1=R_2=H



162 cyclocurcumin



166 dihydroguaiaretic acid



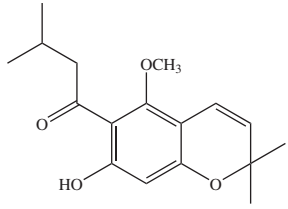
163 malabaricone A R_1=R_2=H
164 malabaricone B R_1=H, R_2=OH
165 malabaricone C R_1=R_2=OH

Some phenylpropanoid derivatives have been screened to have antinematodal activity. Two chromenes rhynchonin A (**167**) and rhynchonin B (**168**) were isolated from the aquatic tropical plant *Rhycholacis penicillata* (Podostemaceae) with

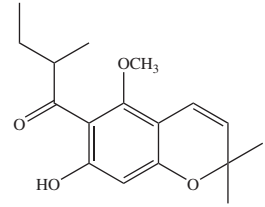
activity against *C. elegans* [121]. The seeds of celery (*Apium graveolens*, Umbelliferae) yielded 3-n-butyl-4,5-dihydrophthalide (**169**), which induced 100% nematode mortality at 12.5μg/ml on *P. redivivus*, and at 50μg/ml on *C. elegans* [122].

In an examination of heartwood and bark parts from three pinaceous species (*P. massoniana, Pinus strobes*, and *Pinus palustris*) resistant to *B. xylophilus*, two lignans, (−)-nortrachelogenin (**170**) and (+)-pinoresinol (**171**), along with two phenylpropanoid derivatives namely methyl ferulate (**172**) and pinosylvin monomethyl ether (**173**) were discovered to have antinematodal activity on *B. xylophilus* [74].

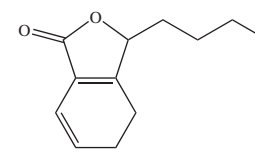
Six lignans namely bursehernin (**174**), matairesinol (**175**), syringaresinol (**176**), buplerol (**177**), guayarol (**178**), and nortrachelogenin triacetate (**179**) from *Bupleurum salicifolium* (Umbelliferae) were tested for their nematostatic activity on the cysts and freed second-stage juveniles of the potato cyst nematodes *G. rostochiensis* and *Globadera pallida*. Among them, bursehernin (**174**) and matairesinol (**175**) at 50μg/ml showed the greatest activities against cyst hatching [123].



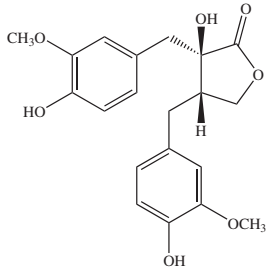
167 rhynchonin A



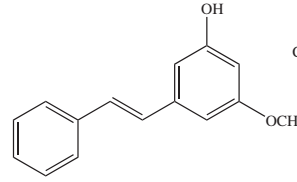
168 rhynchonin B



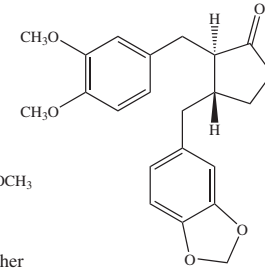
169 3-n-butyl-4,5-dihydrophthalide



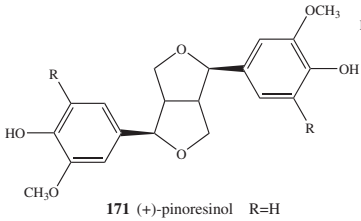
170 (-)-nortrachelogenin



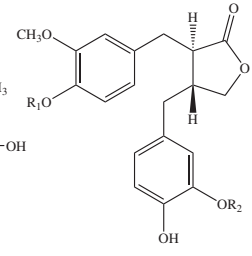
173 pinosylvin monomethyl ether



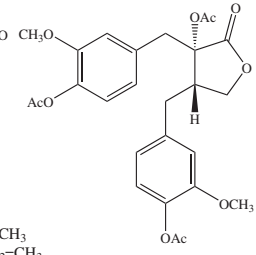
174 bursehernin



171 (+)-pinoresinol R=H
176 syringaresinol R=OCH_3



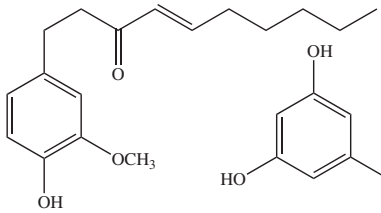
175 matairesinol R_1=H; R_2=CH_3
177 buplerol R_1=CH_3; R_2=CH_3
178 guayarol R_1=CH_3; R_2=H



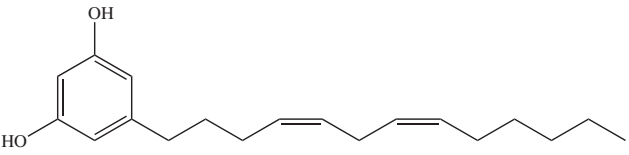
179 nortrachelogenin triacetate

Zingiber officinale (Zingiberaceae) has been eaten along with raw fish and used in traditional Chinese medicine. The extract from this plant exhibited strong inhibitory activity on *Anisakis* sp. larvae *in vitro*. The active compounds were identified as [6]-shogaol (**180**) and [6]-gingerol (**181**). In a solution containing [6]-shogaol (**180**) (62.5μg/ml), more than 90% of larvae lost spontaneous movement within 4h and were destroyed completely within 16h. Microscopic examinations showed destruction of the digestive tract and disturbances of the cuticle [6]-Gingerol (**181**) showed inhibitory activity on *Anisakis* larvae at 250μg/ml. Synergistic effects between [6]-gingerol (**181**) and a small amount of [6]-shogaol (**180**) were observed [124].

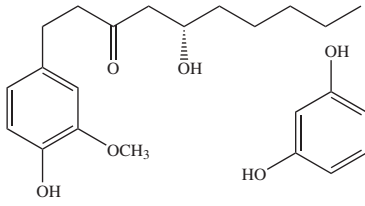
Four nematicidal alkylene resorcinols named as (Z,Z)-5-(trideca-4,7-dienyl) resorcinol (**182**), (Z,Z,Z)-5-(trideca-4,7,10-trienyl)-resorcinol (**183**), (Z,Z,E)-5-(trideca-4,7,10-trienyl) resorcinol (**184**), and (Z)-5-(trideca-4-enyl) resorcinol (**185**), were isolated from *Lithraea molleoides* (Anacardiaceae). They exhibited strong paralyzing effects on the nematode *C. elegans* at a concentration range of 6–50μg/ml [125].



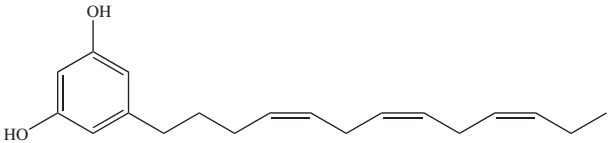
180 [6]-shogaol



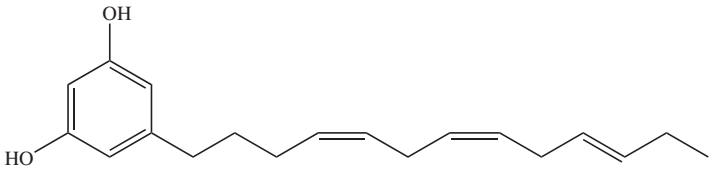
182 (Z,Z)-5-(trideca-4,7-dienyl)resorcinol



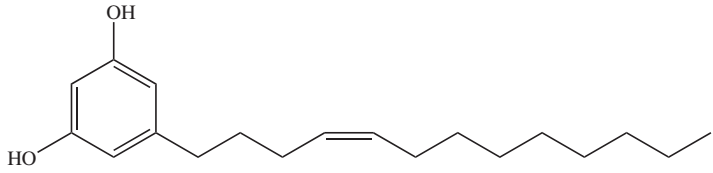
181 [6]-gingerol



183 (Z,Z,Z)-5-(trideca-4,7,10-trienyl)-resorcinol



184 (Z,Z,E)-5- (trideca-4,7,10-trienyl) resorcinol



185 (Z)-5-(trideca-4-enyl)resorcinol

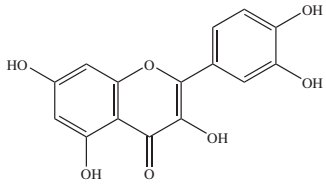
FLAVONOIDS

The flavonoids constitute a broad class of products of the phenylpropanoid acetate pathway. They have a C_{15} skeleton and include a chroman ring bearing an aromatic in positions 2 and 3. Up to now, the isolated antinematodal flavonoids include flavones, flavonols, and isoflavonoids.

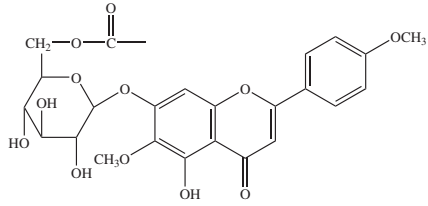
Quercetin (**186**) was a widely distributed flavonol, which inhibited reproduction of *M. javanica* as a soil drench at 400μg/ml [54].

Both lantanoside (**187**) and linaroside (**188**) were two flavone glycosides from the aerial parts of *L. camara* (Verbenaceae), which were lethal to *M. incognita* juveniles at 1.0% [82].

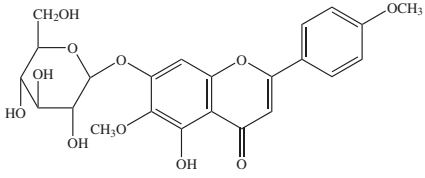
Two nematicidal prenylated flavanones namely 8-(3-methyl-but-2-enyl)-2-phenyl chroman-4-one (**189**) and 2-(4-hydroxyphenyl)-8-(3-methyl-but-2-enyl)-chroman-4-one (**190**) against *M. incognita* and *R. reniformis* were obtained from *Phyllanthus niruri* (Euphorbiaceae) [126].



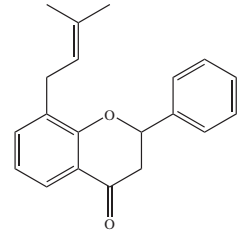
186 quercetin



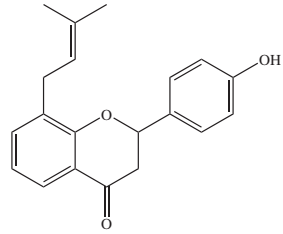
187 lantanoside



188 linaroside



189 8-(3-methyl-but-2-enyl)-2-phenyl chroman-4-one



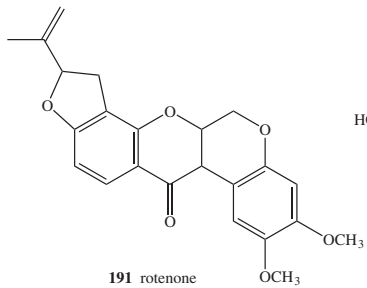
190 2-(4-hydroxyphenyl)-8-(3-methyl-but-2-enyl)-chroman-4-one

Many flavonoids act as phytoalexins, and can be elicited in plants by a variety of factors including viruses, microorganisms and nematodes [127–129]. When resistant plants are infected by nematodes, phytoalexins with antinematodal activity can be produced [127,130].

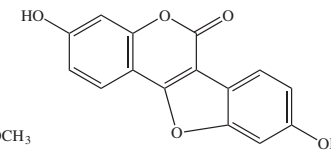
Rotenone (**191**) was reported very early to have antinematodal activity [131]. It displayed nematicidal activity (LC_{90}, 1μg/ml) towards *C. elegans* [132]. Two isoflavonoids coumestrol (**192**) and psoralidin (**193**) as phytoalexins accumulated at the site of nematode attack in lima bean (*Phaseolus lunatus*, Leguminosae) in response to infection by *Pratylenchus scribneri*. Coumestrol (**192**) inhibited motility of *P. scribneri* at 5–25μg/ml, but did not inhibit motility of *M. javanica* [130]. Correlative evidences for a functional role of the related compounds in resistance to nematodes have been obtained [133–135]. In particular, nematode attack on the roots elicits the transcription of genes encoding several enzymes of the shikimate pathway that leads to phytoalexin production [136].

Glyceollin (**194**) as a phytoalexin in soybean (*Glycine max*, Leguminosae) was associated with an incompatible response to root-knot nematodes. It accumulated in soybean roots under challenge from *M. incognita* [137,138], and strongly but reversibly inhibited movement of *M. incognita* [139]. The level of glyceollin (**194**) was found to be related to resistance to the cyst nematode *H. glycines* [140].

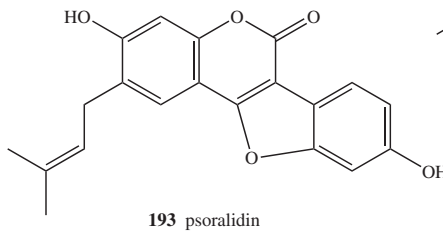
Medicarpin (**195**), which was constitutively expressed in alfalfa (*M. sativa*, Leguminosae), occurred in higher concentrations in resistant alfalfa cultivars, and inhibited motility of *P. penetrans in vitro* [141]. Both medicarpin (**195**) and 4-hydroxymedicarpin (**196**) were isolated as nematicidal principles in roots of Ethiopian medicinal plant *Taverniera abyssinica* (Leguminosae) by virtue of their antinematodal activity in the *C. elegans* bioassay [132].



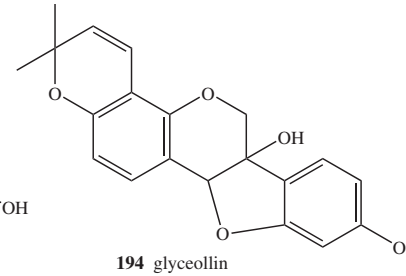
191 rotenone



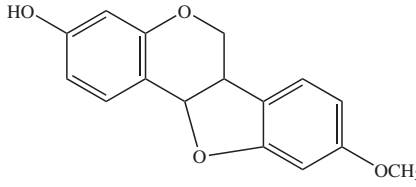
192 coumestrol



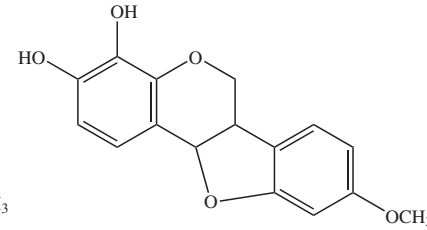
193 psoralidin



194 glyceollin



195 medicarpin

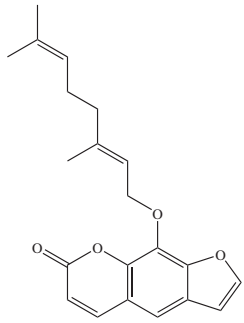


196 4-hydroxymedicarpin

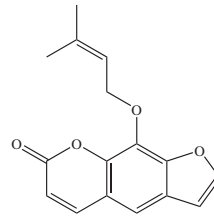
COUMARINS

The coumarins in plants are biosynthesized from *p*-coumaric acid. Three antinematodal coumarins namely 8-geranyloxypsoralen (**197**), imperatorin (**198**), and heraclenin (**199**) were obtained from the extract of *Heracleum candicans* (Umbelliferae). The median lethal concentrations (LC_{50}) of the compounds at 72h were 188.3µg/ml, 161.7µg/ml and 114.7µg/ml, respectively against *B. xylophilus* [142].

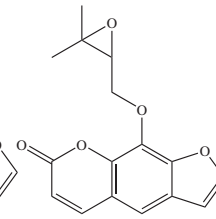
Activity-guided fractionation from the methanol extract of *Ageratum conyzoides* (Compositae) using pine wood nematode *B. xylophilus* successfully led to the isolation and characterization of the nematicidal coumarin (**200**) with LC_{50} value of 75g/cotton ball [143].



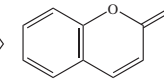
197 8-geranyloxypsoralen



198 imperatorin



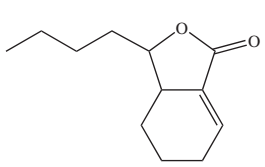
199 heraclenin



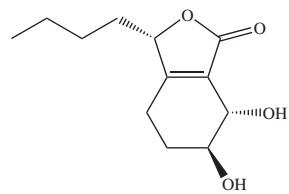
200 coumarin

LACTONES

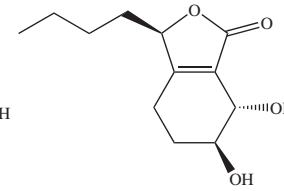
Three lactones isolated from the methanolic extract of *A. graveolens* (Umbelliferae) seeds were identified as sedanolide (**201**), senkyunolide N (**202**), and senkyunolide J (**203**). They showed mosquitocidal, nematicidal, and antifungal activities. Among them, sedanolide (**201**) had the strongest nematicidal activity at 50μg/ml with 100% mortality on the nematode *C. elegans* [144].



201 sedanolide



202 senkyunolide N



203 senkyunolide J

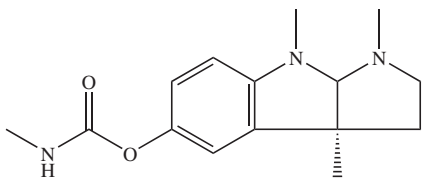
ALKALOIDS

Many plant-derived alkaloids were screened to have antinematodal activity. These antinematodal alkaloids widely distribute in plant species of Leguminosae, Papaveraceae, Liliaceae, Apocyanaceae and Solanaceae [9,145,146].

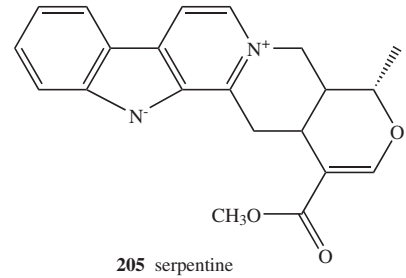
Indole Alkaloids

Physostigmine (**204**), a tricyclic carbamate originally isolated from the Calabar bean (*Physostigma venenosum*, Leguminosae), was the first alkaloid with the reversible acetylcholinesterase inhibitory activity against nematodes. It reversibly immobilized *D. dipsaci* at 1000μg/ml. Interestingly, the pea seedlings treated with much smaller quantities (e.g., 30μg/ml) of physostigmine (**204**) sulphate were significantly protected against subsequent infection of nematodes [147].

Serpentine (**205**) from the roots of *Catharanthus roseus* (Apocyanaceae) induced death and inhibited hatching of *M. incognita* at 0.2% [148], and inhibited subsequent infection of tomato seedlings by *M. incognita* if the seeds were treated with serpentine at 0.2% (**205**) [60].



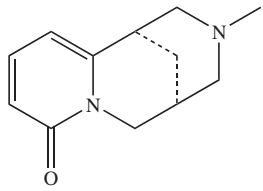
204 physostigmine



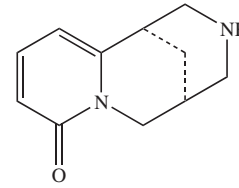
205 serpentine

Quinolizidine Alkaloids

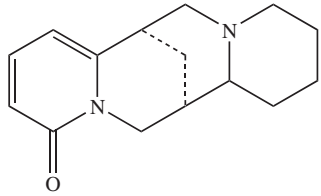
Five quinolizidine alkaloids: *N*-methylcytisine (**206**), anagyrine (**207**), matrine (**208**), sophocarpine (**209**), and sophoramide (**210**) were isolated from the roots of *Sophora flavescens* (Leguminosae) with nematicidal activity against *Bursaphelenchus oxylophilus*. *N*-methylcytisine (**206**) was twice as active as anagyrine (**207**) but only half as nicotine (**223**). All the alkaloids (3–6μg/ml) inhibited reproduction of *B. oxylophilus* in the cotton balls assay [149,150]. Matrine (**208**), cytisine (**211**), *N*-methylcytisine (**206**), and aloperine (**212**) were similarly isolated from *Sophora alopecuroides* as active compounds against *B. xylophilus* [151].



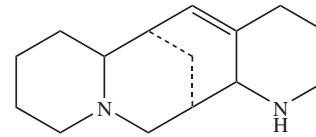
206 *N*-methycytisine



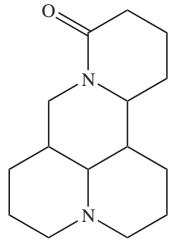
211 cytisine



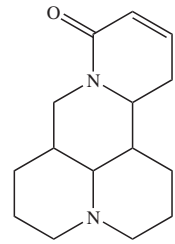
207 anagyrine



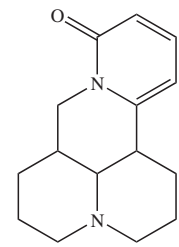
212 aloperine



208 matrine



209 sophocarpine



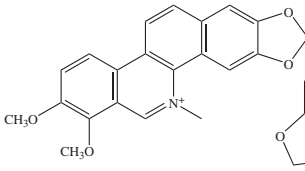
210 sophoramine

Isoquinoline Alkaloids

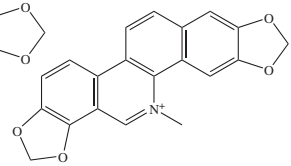
Three isoquinoline alkaloids namely chelerythrine (**213**), sanguinarine (**214**), and bocconine (**215**) from *Bocconia cordata* (also named as *Macleaya cordata*, Papaveraceae) were nematotoxic at 50–100μg/ml against the free-living nematodes *Rhabditis* sp. and *Panagrolaimus* sp. [152,153]. Satou *et al.* examined

protopine (**216**) from the aerial parts of *M. cordata* (Papaveraceae), D-corydaline (**217**) from the aerial parts of *Chelidonium majus* (Papaveraceae), and L-stylopine (**218**) from the air-dried tubers of *Corydalis turtschaninovii* (Papaveraceae) which exhibited strong nematocidal activity against the third-stage larvae of *Strongyloides ratti* and *S. venezuelensis* in humans [154].

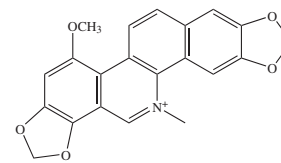
Five alkaloids, sanguinarine (**214**), protopine (**216**), cryptopine (**219**), β-allocryptopine (**220**), and 6-methoxy dihydro chelerythrine (**221**), were bioassay-guided and fractionated from *Macleaya microcarpa* (Papaveraceae). The LC_{50} values for the five compounds were 0.37, 3.31, 4.64, 8.13 and 3.63mg/l, respectively, against *Dactylogyrus intermedius* in Gold fish (*Carassius auratus*) [155].



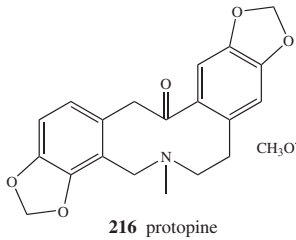
213 chelerythrine



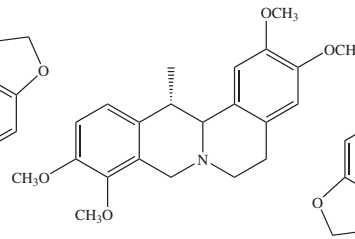
214 sanguinarine



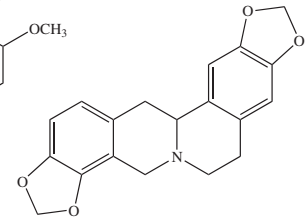
215 bocconine



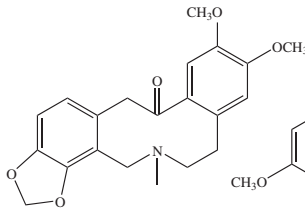
216 protopine



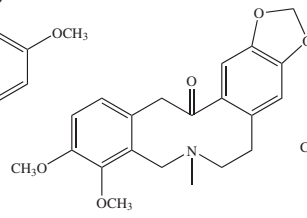
217 D-corydaline



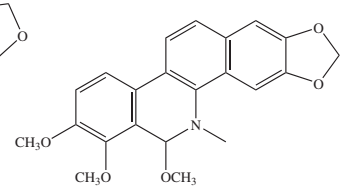
218 L-stylopine



219 cryptopine



220 β-allocryptopine



221 6-methoxy dihydro chelerythrine

Pyrrolidine Alkaloids

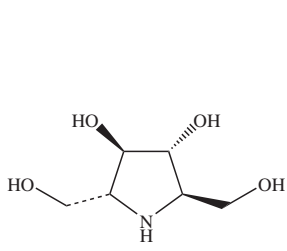
(2R,3R,4R,5R)-2,5-Bis (hydroxymethyl) pyrrolidine-3,4-diol (abbreviated as DMDP, **222**), a pyrrolidine alkaloid isolated from tropical legumes of the *Lonchocarpus* and *Derris* genera, exhibited a range of activities against several

plant-parasitic nematode species. DMDP inhibited cyst hatching of *G. pallida* and immobilized juveniles of *G. rostochiensis*. DMDP also reduced root galling by *Meloidogyne* spp. when it was used as the root drench or seed dressing, or applied as a foliar spray. This indicated that DMDP (**222**) should be a phloem-mobile compound [156].

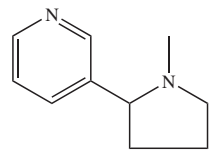
Nicotine (**223**) strongly inhibited hatching of cysts of *H. glycines*, and eggs of *Meloidogyne hapla* and *M. incognita*, but strongly stimulated hatching of cysts of *Heterodera schachtii* [157].

Monocrotaline (**224**), an ornithine-derived pyrrolizidine alkaloid from *Crotalaria spectabilis* (Leguminosae) inhibited movement of *M. incognita* juveniles at 10μg/ml. Exposure of juveniles to monocrotaline solution did not prevent infection, however, there was no correlation between monocrotaline content of various *Crotalaria* species and resistance to *M. incognita* [158].

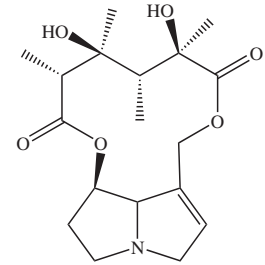
Four pyrrolizidine alkaloids, monocrotaline (**224**), heliotrine (**225**), lasiocarpine (**226**), and senecionine (**227**), from *Crotalaria* sp. (Leguminosae), *Ageratum* sp. (Compositae), *Chromolaena odorata* (Compositae), *Senecio jacobaea* (Compositae) were examined to show nematicidal, ovicidal and repellent effects on different nematodes such as *M. incognita*, *H. schachtii*, *P. penetrans*, *Phasmarhabditis hermaphrodita*, and *Rhabditis* sp. [159,160].



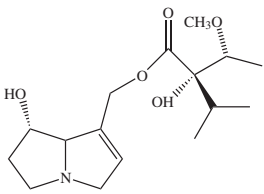
222 DMDP



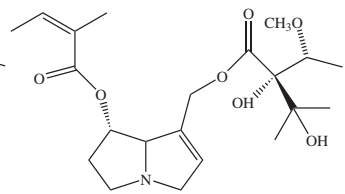
223 nicotine



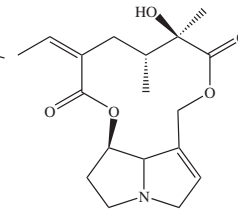
224 monocrotaline



225 heliotrine



226 lasiocarpine



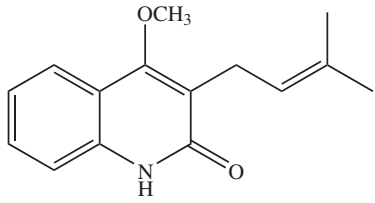
227 senecionine

Other Alkaloids

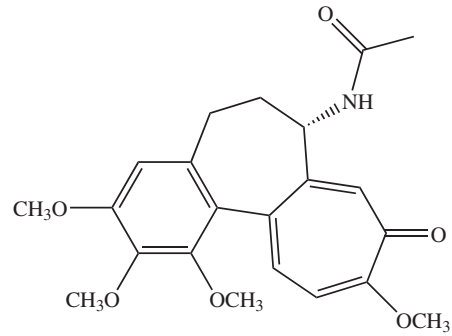
The extract from the dried unripe fruits of the Chinese medicinal plant *Evodia rutaecarpa* (Rutaceae) was bioassay-guided and fractionated using larvae of

Schistosoma mansoni, *Ostertagia circumcincta*, and *C. elegans*. The antinematodal component was identified as atanine (**228**), an anthranilic acid-derived quinolone alkaloid, which immobilized larvae and adults of *C. elegans* (LC_{50}, 10μg/ml) and J_4 larvae of *O. circumcincta* (LC_{50}, 50μg/ml) [114].

Colchicine (**229**) significantly inhibited the mobility of *H. schachtii* males when pretreated for 2 or 4h at 100mmol/l. The primary targets of colchicine (**229**) should be the microtubules of motor neurons, interneurons or of the cytoskeleton rather than sensory neuron microtubules [161]. High concentrations (i.e., 0.45%) of colchicine (**229**) were nematicidal to *M. incognita* juveniles. The activity of this compound from the seeds of *Gloriosa superba* (Liliaceae) was insufficient to account for the toxicity [162].



228 atanine



229 colchicine

ISOTHIOCYANATES AND GLUCOSINOLATES

Isothiocyanates are amino acid derivatives that contain the monovalent group –NCS. Glucosinolates are β-D-thioglucosides distinguished from one another by differences in their organic side chains, and are grouped as either aliphatic, aromatic, or indole forms. Both isothiocyanates and glucosinolates are mainly produced by cruciferous species. One of the first studied chemically mediated interactions between plants and nematodes was that between mustard plants (*Brassica* spp. and *Sinapis* spp.) and *G. rostochiensis*. A series of investigations led to the discovery of allyl isothiocyanate (**230**), the major component of black mustard (*Brassica nigra*, Cruciferae) seed oil. It inhibited hatching of *Globodeta rostochinensis* eggs at concentrations as low as 1μg/ml [163,164]. Allyl isothiocyanate (**230**) was also toxic to *C. elegans* with LC_{50} value as 40μg/ml that was nearly three orders of magnitude more potent than the corresponding glucosinolate sinigrin (**231**) [165,166]. It is interesting to note that sinigrin (**231**) was used as a soil fumigant, and amoscanate (4-isothiocyanato- N-(4-nitrophenyl)-benzeneamine, **232**) and bitoscanate (1,4-diisothiocyanatobenzene, **233**) as anthelmintics against *Ancylostoma* and *Necator* infections [167].

2-Phenylethyl isothiocyanate (**234**), occurs in *Sinapis alba* (Cruciferae) roots, inhibited egg hatching in laboratory experiments at 50μg/ml and improved yield of potato in field experiments [168].

Chemical investigations of isothiocyanates as nematicides were initiated in 1935 [169]. One of the most successful nematicides was metam sodium (**235**), which degraded in soil to yield methyl isothiocyanate (**236**) [170].

As allyl isothiocyanate (**230**) was not volatile, and did not move within soil either. Modified injection equipment and use of tarpaulins could increase its efficacy [171]. Ally isothiocyanate (**230**) was screened to have selective nematicidal activity on the hatching of the nematode cysts or eggs. At 5μg/ml, it strongly inhibited *H. glycines* and *M. incognita*, no effect on *M. hapla*, but stimulated the hatching of cysts of *H. schachtii* [172].

Benzyl isothiocyanate (**237**) was fractioned from the myrosinase-treated crude extract of papaya (*Carica papaya*, Caricaceae), and was proved to have antinematodal activity [173].

Isothiocyanates display a range of activities towards many soil organisms including nematodes, fungi, viruses, and insects. They are potent electrophiles which react with free amino groups of either amino acids or proteins. Evidence for the involvent of glucosinolate-derived isothiocyanates in toxicity of brassicaceous soil amendments was provided by experiments demonstrating that rapeseed leaf or seed extracts or specific glucosinolates were toxic to *C. elegans*, *Xiphinema americanum*, *H. schachtii*, or *G. rostochinensis* only when the extracts or compounds were enzymatically hydrolyzed [165,174–176]. Rapeseed or canola (*Brassica napus*, Cruciferae) has been received increasing attention as a rotation or green manure crop to provide nematode control, and isothiocyanates are also involved in this toxicity [177].

Virtually all cruciferous species produce thioglucose conjugates called glucosinolates, which are located within the vacuole of each cell. Glucosinolates are considered to have little biological activity themselves. After hydrolysis, they produce compounds which contribute to plant defence. The hydrolysis could be catalysed by endogenous β-thioglucosidases (myrosinases) which are activated on tissue damage, in the case of nematodes, when the stylet perforates the cell. The hydrolysis of glucosinolates initially involves cleavage of the thioglucoside yielding D-glucose and unstable thiohydroximate-*O*-sulphonate that spontaneously rearranges, resulting in the production of sulphate and a range of possible reaction products, thiocyanate, isothiocyanate of nitrile depending on factors such as substrate, pH or the availability of ferrous ions [21,178,179]. The enzymatic hydrolysis of glucosinolates *via* myrosinase is shown in Fig. 1 [180]. Tissues of cruciferous plants containing glucosinolates reduce the number of soil-borne parasitic nematodes, including *Meloidogyne* spp., *H. schachtii*, and the root-lesion nematode *P. penetrans*. Glucosinolates are not active by themselves, but they become active in the presence of β-thioglucosidase. Thus sinigrin (**231**), gluconapin (**238**), glucoraphasatin (**239**), and glucotropaeolin (**240**) (0.5%), with added myrosinase, caused 93–100% mortality of J_2 juvenile of *H. schachtii* [175].

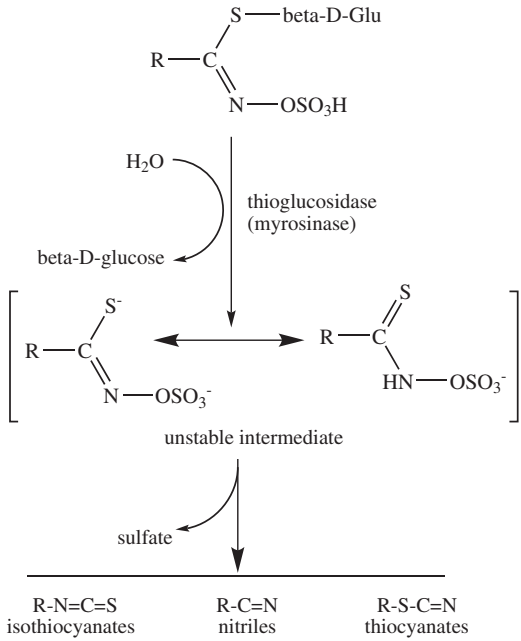


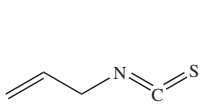
FIGURE 1 Enzymatic hydrolysis of glucosinolates *via* myrosinase [180].

Additional evidence for the involvement of a specific glucosinolate as the determining factor for nematode resistance results from the observation that the ability of *Pratylenchus neglectus* to reproduce on *B. napus* cultivars was correlated with their lower percentages of 2-phenylethyl glucosinolate (**241**), but not total glucosinolates [181]. 2-Phenylethyl isothiocyanate (**241**) applied to soil at 16.2μg/g suppressed reproduction of *P. neglectus* [181]. In contrast, egg production of *M. javanica* was not correlated with root glucosinolate concentrations or compositions in most of the 11 *Brassica* cultivars examined [182]. The conclusion was that although glucosinolates are involved in nematode suppression, other chemical and biological factors should also be involved. The complex chemistry and compartmentalization of the glucosinolates and other sulphur-containing compounds in the Cruciferae and the complexity of nematode feeding may make interspecies comparisons difficult [22].

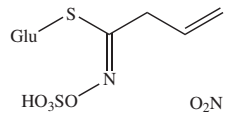
Buskov *et al.* selected eight glucosinolates including allyglucosinolate (sinigrin, **231**), but-3-enylglucosinolate (gluconapin, **238**), benzylglucosinolate (glucotropaeolin, **240**), 2-phenethylglucosinolate (gluconasturtiin, **241**), 4-methylsulfinylbut-3-enylglucosinolate (glucoraphenin, **242**), 4-hydroxybenzylglucosinolate (sinalbin, **243**), (2S)-2-hydroxybut-3-enylglucosinolate (epiprogoitrin, **244**), (2R)-2-hydroxy-2-phenylethylglucosinolate (epi-glucobarbarin, **245**) to test their nematicidal activity against the potato cyst nematode (*G. rostochiensis*). The glucosinolates were used at three concentrations as

0.05, 0.3 and 1.0mg/ml, in the presence or absence of the enzyme myrosinase. No effects of the compounds on the mortality were monitored. However, when active myrosinase was included with 1mg/ml of 2-phenethylglucosinolate (**241**) at pH 6.5, 100% mortality was observed within just 16h. A similar effect was achieved at the same concentration of allylglucosinolate (**231**) or benzylglucosinolate (**238**) in the myrosinase-containing solutions. The aglucone products released from the other glucosinolates were observed to have pronounced effects on the nematodes [183].

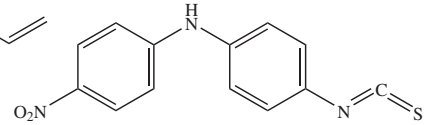
Similarly, Zasada and Ferris compared the nematicidal activities of eight isothiocyanates, i.e., allyl- (**230**), 2-phyenylethyl- (**234**), methyl- (**236**), benzyl- (**237**), butyl- (**246**), ethyl- (**247**), phenyl- (**248**), and 4-methylsulfinyl (butyl)- (**249**) isothiocyanates. The LC_{90} values were 0.01μmol/ml and 0.03μmol/ml for 2-phenylethyl isothiocyanate and 0.48μmol/ml and 0.35μmol/ml for phenyl isothiocyanate (**234**) for *T. semipenetrans* and *M. javanica*, respectively [180]. Cruciferous sources of benzyl (**237**) or 2-phenylethyl (**234**) isothiocyanates are the most promising candidates for plant-parasitic nematode managements [184]. Another report on the nematicidal activity of the hydrolysis products of the tested glucosinolates *via* myrosinase on the root-knot nematode *M. incognita* showed that allyl- (**230**), 2-phenylethyl- (**234**), benzyl- (**237**), and 4-methylsulfinyl (butyl)- (**249**) isothiocyanates exihibted strong activity at a concentration range of 11, 15, 21 and 34μmol/l, respectively [185].



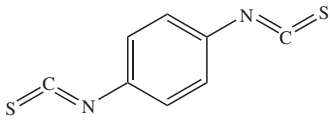
230 allyl isothiocyanate



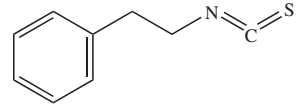
231 sinigrin



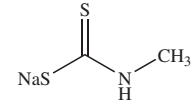
232 amoscanate



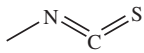
233 bitoscanate



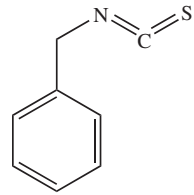
234 2-phenylethyl isothiocyanate



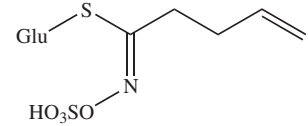
235 metam sodium



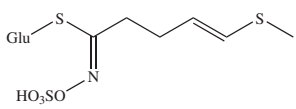
236 methyl isothiocyanate



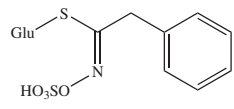
237 benzyl isothiocyanate



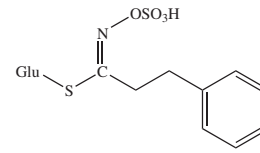
238 gluconapin



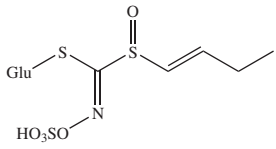
239 glucoraphasatin



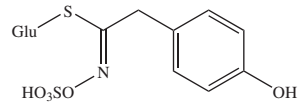
240 glucotropaeolin



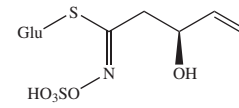
241 2-phenylethyl glucosinolate = gluconasturtin



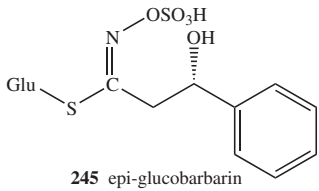
242 glucoraphenin



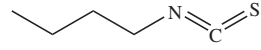
243 sinalbin



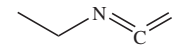
244 epi-progoitrin



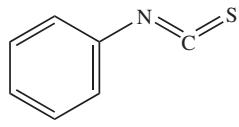
245 epi-glucobarbarin



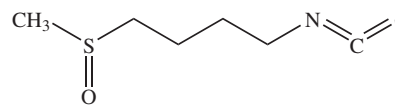
246 butyl isothiocyanate



247 ethyl isothiocyanate



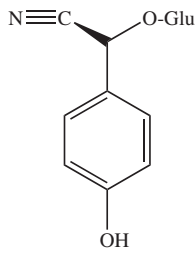
248 phenyl isothiocyanate



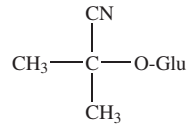
249 4-(methylsulfinyl) butyl isothiocyanate

CYANOGENIC GLYCOSIDES

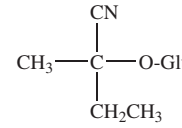
Cyanogenic glucosides are amino acid-derived products [186]. The ability of synthesizing these glucosides is common across many plant genera including several important crop plants like sorghum (*Sorghum bicolor*, Graminae), cassava (*Manihot esculenta*, Euphorbiaceae), flax (*Linum usitatissimum*, Linaceae), and almonds (*Prunus dulcis*, Rosaceae). Degradation of cyanogenic glucosides is catalysed by enzymes and results in release of hydron cyanide which is known to be very toxic to a wide range of organisms, including nematodes [187]. Accordingly, cyanogenic glucosides are classified as phytoanticipins that may play a role in plant defence [188].



250 dhurrin



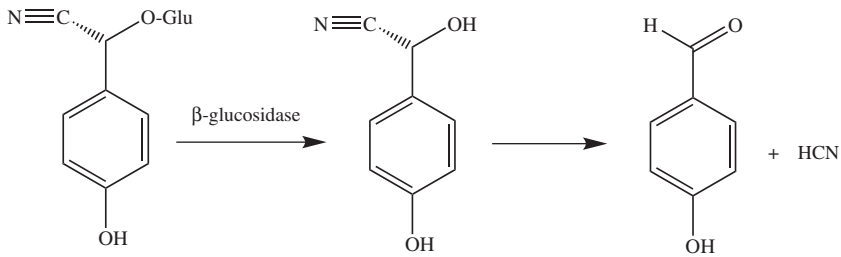
253 linamarin



254 lotaustralin

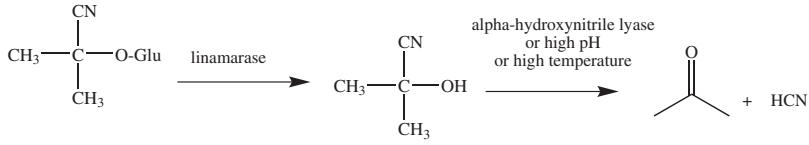
For example, the green manures of Sudan grass (*Sorghum sudanense*, Graminae) have been used as the nematode suppressants [189]. Common sorghum (*S. bicolor*, Graminae) also had the similar effects on nematodes [190]. They contain a cyanogenic glycoside dhurrin (**250**), which can be hydrolyzed by β-glucosidase, and releases an intermediate *p*-hydroxymandelonitrile (**251**), which then dissociates to produce free hydrogen cyanide (HCN) and *p*-hydroxybenzaldehyde (*p*-HBA, **252**) shown in Fig. 2 [191]. As the antinematodal activity of the fractionated Sudan grass extracts against *M. hapla* is associated with the presence of hydrogen cyanide in the fractions, it seems that dhurrin (**250**) is involved in the mode of action of Sudan grass on *M. hapla* [189,192].

The roots of cassava (*M. esculenta*, Euphorbiaceae) contain various quantities of cyanogenic glucosides, particularly linamarin (**253**) and lotaustralin (**254**) [193,194]. These compounds, and the enzymes involved in their catabolism, namely linamarase (EC 3.2.1.21) and α-hydroxynitrile lyase (EC 4.1.2.37), are known to reside in different subcellular compartments. When the cells are damaged, the enzymes hydrolyze these glucosides, releasing cyanide *via* cyanohydrin intermediates. The breakdown of linamarin by the enzyme linamarase is very rapid, releasing glucose and an intermediate acetone cyanohydrin (**255**). At high pH, or at high temperatures, the cyanohydrin decomposes spontaneously, producing acetone together with volatile hydrogen cyanide which is very toxic to organisms [195]. The catabolism of linamarin (**253**) to hydrogen cyanide and acetone *via* acetone cyanohydrin is shown in Fig. 3. Manipueira, the liquid



250 dhurrin **251** *p*-hydroxymandelonitrile **252** *p*-hydroxybenzaldehyde

FIGURE 2 Breakdown of dhurrin to the intermediate *p*-hydroxymandelonitrile and the final products of *p*-hydroxybenzaldehyde and hydrogen cyanide [189].



253 linamarin **255** acetone cyanohydrin acetone

FIGURE 3 Catabolism of linamarin to hydrogen cyanide and acetone *via* acetone cyanohydrin [195].

formed during processing of cassava roots, has been utilized for nematode control for decades in Brazil [196].

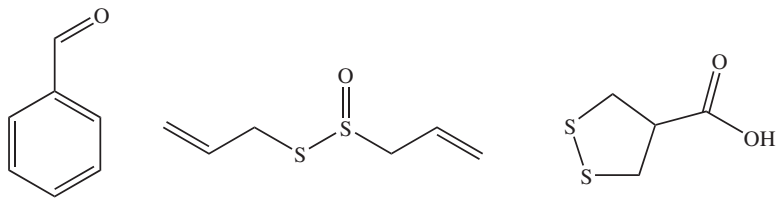
OTHER COMPOUNDS

The sap of *K. hookeriana* (Myristicaceae) was particularly active against *B. xylophilus* in a cotton ball bioassay. The isolated benzaldehyde (**256**) inhibited *M. incognita*-induced root gall formation in greenhouse or microplot studies in cotton, in search of biologically based fumigants [48].

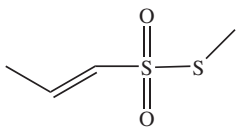
Allicin (**257**), a major component of garlic (*Allium sativum*, Liliaceae), inhibited hatching of *M. incognita* at concentration of 0.5μg/ml, and was toxic to juveniles at 2.5μg/ml [197]. Immersion of tomato roots in allicin solutions as a prophylactic measure was beset by problems of phytotoxicity and lack of nematotoxicity, but a 5-min immersion in 25μg/ml of allicin inhibited penetration of the juveniles in roots by 50%, and was not phytotoxic [197].

Asparagusic acid (**258**), a sulphur-containing compound, was identified in the roots of asparagus (*Asparagus officinalis*, Liliaceae) as a nematicide. This compound inhibited hatching of *H. glycines* and *G. rostochinensis*, and induced mortality in three other nematode species at 50μg/ml [198].

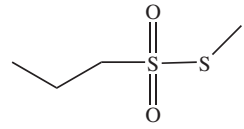
Other sulphur-containing nematicidal substances include allygrin (**259**) from *A. grayi*, and five compounds from the ether extract of *Allium fistulosum* var. *caespitosum*, which were S-methyl propanethiosulfonate (**260**), dimethl trisulfide (**261**), methyl propyl trisulfide (**262**), dipropyl disulfide (**263**), and allylpropyl disulfide (**264**). These compounds showed strong nematicidal activity against *M. incognita* [27].



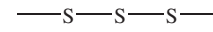
256 benzaldehyde **257** allicin **258** asparagusic acid



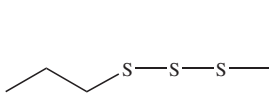
259 allygrin



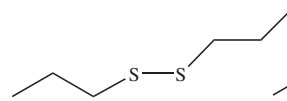
260 S-methyl propanethiosulfonate



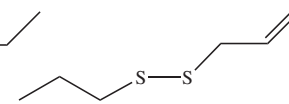
261 dimethyl trisulfide



262 methyl propyl trisulfide



263 dipropyl disulfide



264 allylpropyl disulfide

CONCLUDING REMARKS

The control of plant-parasitic nematode diseases is of great importance due to their widespread presence and massive effects on the health of plants, animals and human beings. If a certain nematocide has been used for a long time, resistant varieties of nematodes will probably appear. Environment damage and human health will become great problems. Therefore, searching for new nematicides and control strategies is always necessary [22]. In agricultural practise, the use of plants and plant extracts is one of the promising methods for nematode control. They are cheap, easy to apply, produce no pollution hazards, and have the capacity to structurally and nutritionally improve the soil health.

In search of alternatives to chemically control nematodes, a number of plant secondary metabolites with potential antinematodal activity have been studied and clarified. Generally, the development of plant-derived antinematodal compounds has not yet reached maturity [22]. Efforts have largely consisted of basic and descriptive research. One conspicuous aspect of this descriptive research is that only a small number of plant families have been investigated. Obviously, a wide range of plant taxa need to be included in the future. In addition, most plant nematological studies have started with plants or plant secondary metabolites known to be active against other pest and pathogens. The action modes of most nematicidal plant metabolites are still unknown. Indeed, in some cases the concentrations evaluated *in vitro* have been so high that physiological activity would be contraindicated. In such experiments, osmotic, pH, and other nonspecific effects cannot be excluded [22].

There is less interest among the public at large in mode of action than in the development of environmentally safe, inexpensive, agronomically useful compounds [95,199]. Are nematode-antagonistic phytochemicals likely to be safe, effective, and useful? An argument can be made that naturally occurring compounds are often more readily degraded in the environment than synthetic compounds. With respect to efficacy, only a few compounds have exhibited activity as effective as commercial nematicides. Although efficacy of specific phytochemicals may be high *in vitro* against some developmental stages of

some nematodes, the behaviour of these compounds in soil as well as other considerations have limited their application in agriculture [22]. Furthermore, the cost of purification and preparation of the nematicidal compounds from plants should be considered.

The bioassays selected in phytochemical research are critical. Few investigators have utilized more than one nematode species in bioassays. Direct *in vitro* tests must be complemented by *in vivo*, soil-based experiments in order to examine phytotoxicity or binding to the soil. In order to determine whether a purified phytochemical is actually nematode-antagonistic or not, results should be duplicated, and its antinematodal activity should be compared with a chemically synthesized compound [22].

In a word, discovery of novel antinematodal metabolites from plants will provide new knowledge for use of natural products in plant protection. Agricultural development and utilization of plant-derived antinematodal metabolites with low mammalian and environmental toxicity will guarantee food safety and agriculture sustainability, and ensure a safe and healthy environment. Investigation on antinematodal phytochemicals should be a promising area in nematode management.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the grants from the Hi-Tech R&D Program of China (2011AA10A202 and 2006AA10Z423), the National Basic Research Program of China (2010CB126105), and the Special Fund for Agro-Scientific Research in the Public Interest of China (200903052).

REFERENCES

[1] S.R. Koenning, C. Overstreet, J.W. Noling, P.A. Donald, J.O. Becker, B.A. Fortnum, Suppl. J. Nematol. 31 (1999) 587–618.
[2] I.N. Kashaija, B.D. Mcintyre, H. Ssali, F. Kizito, Nematology 6 (2004) 7–12.
[3] S.M. Shneider, E.N. Rosskopf, J.G. Leesch, D.O. Chellemi, C.T. Bull, M. Mazzola, Pest Manag. Sci. 59 (2003) 814–826.
[4] C.R.R. Hooks, K.-H. Wang, A. Ploeg, R. McSorley, Appl. Soil Ecol. 46 (2010) 307–320.
[5] P. Crews, L.M. Hunter, In: D.H. Attaway, O.R. Zaborsky (Eds.), Marine Biotechnology: Pharmaceutical and Bioactive Natural Products, Plenum Press, New York, 1992, pp. 117–158.
[6] H. Anke, O. Sterner, Curr. Org. Chem. 1 (1997) 361–374.
[7] J.M. Halbrendt, J. Nematol. 28 (1996) 8–14.
[8] B.S. Sipes, A.S. Arakaki, Suppl. J. Nematol. 29 (1997) 721–724.
[9] B. Meera, S. Kumar, S.B. Kalidhar, J. Med. Aromat. Plant Sci. 25 (2003) 441–465.
[10] D.J. Chitwood, In: S.O. Duke, J.J. Menn, J.R. Plimmer (Eds.), Pest control with Enhanced Environmental Safety, American Chemical Society, Washington, DC, 1993, pp. 300–315.
[11] M. Akhtar, I. Mahmood, Bioresour. Technol. 48 (1994) 189–201.
[12] E. Riga, C. Hooper, J. Potter, Phytoprotection 86 (2005) 31–35.
[13] F. Kiuchi, Nat. Med. 49 (1995) 364–372.
[14] M.B. Isman, Crop Prot. 19 (2000) 603–608.

[15] M.R.J.R. Albuquerque, S.M.O. Costa, P.N. Bandeira, G.M.P. Santiago, M. Andrade-Neto, E.R. Silveira, et al., Anais da Academia Brasileira de Ciencias 79 (2007) 209–213.
[16] J. Kim, S.-M. Seo, S.-G. Lee, S.-C. Shin, I.-K. Park, J. Agric. Food Chem. 56 (2008) 7316–7320.
[17] M. Zouhar, O. Douda, D. Lhotsky, R. Pavela, Plant Prot. Sci. 45 (2009) 66–73.
[18] P. Barbosa, A.S. Lima, P. Vieira, L.S. Dias, M.T. Tinoco, J.G. Barroso, L.G. Pedro, A.C. Figueiredo, M. Mota, J. Nematol. 42 (2010) 8–16.
[19] R. Cetintas, M.M. Yarba, J. Anim. Vet. Adv. 9 (2010) 222–225.
[20] N.G. Ntalli, F. Ferrari, I. Giannakou, U. Menkissoglu-Spiroudi, J. Agric. Food Chem. 58 (2010) 7856–7863.
[21] E.L. Ghisalberti, In: Attaur-Rahman (Ed.), Studies in Natural Products Chemistry, vol. 26, Elsevier Science B.V., Amsterdam, 2002, pp. 425–506.
[22] D.J. Chitwood, Annu. Rev. Phytopathol. 40 (2002) 221–249.
[23] P. Ohri, S.K. Pannu, J. Environ. Res. Dev. 4 (2009) 171–177.
[24] K. Munakata, In: D.L. Whitehead, W. Bowers (Eds.), Natural Products for Innovatvie Pest Management, Current Themes in Tropical Science, vol. 2, Pergamon Press, Oxford, 1983, pp. 299–309.
[25] R.M. Sayre, Z.A. Patrick, H.J. Thorpe, Nematologica. 11 (1965) 263–268.
[26] N.G. Ntalli, S. Vargiu, U. Menkissoglu-Spiroudi, P. Caboni, J. Agric. Food Chem. 58 (2010) 11390–11394.
[27] M. Tada, Y. Hiroe, S. Kiyohara, S. Suzuki, J. Agric. Food Chem. 52 (1988) 2383–2385.
[28] Y. Kimura, M. Mori, S.-B. Hyeon, A. Suzuki, Y. Mitsui, Agric. Biol. Chem. 45 (1981) 249–251.
[29] M.A. Saleh, F.H. Abdel Rahman, N.A. Ibrahim, N.M. Taha, J. Chem. Ecol. 13 (1987) 1361–1370.
[30] E.L. Davis, D.M. Meyers, C.J. Dullum, Suppl. J. Nematol. 29 (1997) 677–684.
[31] M.A. Nogueira, J.S. de Oliverira, S. Ferraz, Phytochemistry 42 (1996) 997–998.
[32] M.A. Noguerira, J.S. de Oliveira, S. Ferraz, M.A. Dos Santos, Nematol. Medit. 24 (1996) 249–252.
[33] S.M. Seo, J. Kim, E. Kim, H.-M. Park, Y.-J. Kim, I.-K. Park, J. Agric. Food Chem. 58 (2010) 1823–1827.
[34] F.J. Gommers, Helminthol. Abstr. 50B (1981) 9–14.
[35] K. Kawazu, Y. Nishii, S. Nakajima, Agric. Biol. Chem. 44 (1980) 903–906.
[36] Y. Kimura, M. Mori, A. Suzuki, A. Kobayashi, Agric. Biol. Chem. 45 (1981) 2915–2917.
[37] S. Kogiso, K. Wada, K. Munakata, Agric. Biol. Chem. 40 (1976) 2085–2089.
[38] S. Kogiso, K. Wada, K. Munakata, Tetrahedron Lett. 2 (1976) 109–110.
[39] S. Sanchez de Viala, B.B. Brodie, E. Rodriguez, D.M. Gibson, J. Nematol. 30 (1998) 192–200.
[40] D.A. Rickard, A.W. Dupree, J. Nematol. 10 (1978) 296–297.
[41] L.T. Hoan, R.G. Davide, Philipp. Agric. 62 (1979) 285–295.
[42] M.A. Siddiqui, M.M. Alam, Biol. Wastes 21 (1987) 221–229.
[43] J.H. Uhlenbroek, J.D. Bijloo, Rec. Trav. Chim. Pays-Bas. 77 (1958) 1004–1009.
[44] J.H. Uhlenbroek, J.D. Bijloo, Rec. Trav. Chim. Pays-Bas. 78 (1959) 382–390.
[45] F.J. Gommers, D.J.M. Voor in Tholt, Neth. J. Plant Pathol. 82 (1976) 1–8.
[46] D.H.S. Horn, J.A. Lamberton, Aust. J. Chem. 16 (1963) 475–479.
[47] I. Marotti, M. Marotti, R. Piccaglia, A. Nastri, S. Grandi, G. Dinelli, J. Sci. Food Agric. 90 (2010) 1210–1217.
[48] E.M. Bauske, R. Rodriguez-Kabana, V. Estaun, J.W. Kloepper, D.G. Robertson, C.F. Weaver, P.S. King, Nematropica. 24 (1994) 143–150.
[49] F.Q. Alali, X.-X. Liu, J.L. McLaughlin, J. Nat. Prod. 62 (1999) 504–540.

[50] J.K. Rupprecht, Y.-H. Hui, J.L. Mclaughlin, J. Nat. Prod. 53 (1990) 237–278.
[51] M.C.M. Torres, J.C. Assuncao, G.M.P. Santiago, M. Andrade-Neto, E.R. Silveira, L.V. Costa-Lotufo, D.P. Bezerra, F. Marinho, D.B. Jose, F.A. Viana, O.D.L. Pessoa, Chem. Biodivers. 5 (2008) 2724–2728.
[52] A. Soler-Serratosa, N. Kokalis-Burelle, R. Rodriguez-Kabana, C.F. Weaver, P.S. King, Nematropica. 26 (1996) 57–71.
[53] M. Ishikawa, Y. Shuto, H. Watanabe, Agric. Biol. Chem. 50 (1986) 1863–1866.
[54] A. Osman, D.R. Viglierchio, Rev. Nematol. 11 (1988) 93–98.
[55] D.R. Viglierchio, F.F. Wu, Nematropica. 19 (1989) 75–79.
[56] N.K. Sangwan, B.S. Verma, K.K. Verma, K.S. Dhinda, Pest. Sci. 28 (1990) 331–335.
[57] M.S. Malik, N.K. Sangwan, K.S. Dhindsa, K.K. Verma, D.S. Bhatti, Pesticides 21 (1987) 30–32.
[58] Y. Oka, S. Nacar, E. Putievsky, U. Ravid, Z. Yaniv, Y. Spiegel, Phytopathology 90 (2000) 710–715.
[59] N.K. Leela, R.M. Khan, P.P. Reddy, E.S.J. Nidiry, Nematol. Medit. 20 (1992) 57–58.
[60] M.S. Rao, P.P. Reddy, A. Mittal, M.V. Chandravadana, M. Nagesh, Nematol. Medit. 24 (1996) 49–51.
[61] N. Gokte, M.L. Maheshwari, V.K. Mathur, Indian J. Nematol. 21 (1991) 123–127.
[62] D. Kumar, S.K. Mishra, S.K. Tandan, H.C. Tripathi, Indian J. Pharmacol. 27 (1995) 161–166.
[63] S. Echeverrigaray, J. Zacaria, R. Beltrao, Phytopathology 100 (2010) 199–203.
[64] N.G. Ntalli, F. Ferrari, I. Giannakou, U. Menkissoglu-Spiroudi, Pest Manag. Sci. 67 (2011) 341–351.
[65] J. Lei, M. Leser, E. Enan, Biochem. Pharmacol. 79 (2010) 1062–1071.
[66] J.A. Veech, M.A. McClure, J. Nematol. 9 (1977) 225–229.
[67] J.A. Veech, J. Nematol. 11 (1979) 240–246.
[68] N. Khoshkoo, P.A. Hedin, J.C. McCarty, J. Agric. Food Chem. 42 (1994) 204–208.
[69] V. Samoylenko, D.C. Dunbar, M.A. Gafur, S.I. Khan, S.A. Ross, J.S. Mossa, et al., Phytother. Res. 22 (2008) 1570–1576.
[70] R. Mahajan, P. Singh, K.L. Bajaj, P.S. Kalsi, Nematologica. 32 (1986) 119–123.
[71] A.E. Desjardins, S.P. McCormick, R.L. Plaisted, B.B. Brodie, J. Agric. Food Chem. 45 (1997) 2322–2326.
[72] S.V. Zinoveva, L.I. Chalova, Helminthologia. 24 (1987) 303–309.
[73] T.J.W. Alphey, W.M. Robertson, G.D. Lyon, Rev. Nematol. 11 (1988) 399–404.
[74] T. Suga, S. Ohta, K. Munesada, N. Ide, M. Kurokawa, M. Shimizu, E. Ohta, Phytochemistry 33 (1993) 1395–1401.
[75] M. Heinrich, J.E. West, B.R. Ortiz de Montellano, E. Rodriguez, Annu. Rev. Pharmacol. Toxicol. 38 (1998) 539–565.
[76] E. Van den Enden, Expert Opin. Pharmacol. 10 (2009) 435–451.
[77] L. Hong, G. Li, W. Zhou, X. Wang, K. Zhang, Pest Manag. Sci. 63 (2007) 301–305.
[78] S. Kogiso, K. Wada, K. Munakata, Agric. Biol. Chem. 40 (1976) 2119–2120.
[79] J.-X. Shi, Z.-X. Li, T. Nitoda, M. Izumi, H. Kanzaki, N. Baba, K. Kawazu, S. Nakajima, Biosci. Biotechnol. Biochem. 71 (2007) 1086–1089.
[80] S. Sinha, U. Chakraborty, S.D. Mishra, B.S. Parmar, Indian J. Nematol. 35 (2005) 183–186.
[81] V. Sharma, S. Walia, J. Kumar, M.G. Nair, B.S. Parmar, J. Agric. Food Chem. 51 (2003) 3966–3972.
[82] S. Begum, A. Wahab, B.S. Siddiqui, F. Qamar, J. Nat. Prod. 63 (2000) 765–767.
[83] S. Begum, S.Q. Zehra, B.S. Siddiqui, S. Fayyaz, M. Ramzan, Chem. Biodivers. 5 (2008) 1856–1866.

[84] F. Qamar, S. Begum, S.M. Raza, A. Wahab, B.S. Siddiqui, Nat. Prod. Res. 19 (2005) 609–613.
[85] C.J. Njoku, L. Zeng, I.U. Asuzu, N.H. Oberlies, J.L. Mclaughlin, Int. J. Pharmacogn. 35 (1997) 134–137.
[86] M.P. Argentieri, T. D'Addabbo, A. Tava, A. Agostinelli, M. Jurzysta, P. Avato, Eur. J. Plant Pathol. 120 (2008) 189–197.
[87] T. D'Addabbo, P. Avato, A. Tava, Eur. J. Plant Pathol. 125 (2009) 39–49.
[88] T. D'Addabbo, T. Carbonara, P. Leonetti, V. Radicci, A. Tava, P. Avato, Phytochem. Rev. 10, 2011, doi:10.1007/s11101-010-9180-2.
[89] Z. Guo, S. Vangapandu, R.W. Sindelar, L.A. Walker, R.D. Sindelar, Curr. Med. Chem. 12 (2005) 173–190.
[90] I.J.C. Vieira, R. Braz-Filho, In: Attaur-Rahman (Ed.), Studies in Natural Products Chemistry, vol. 33, Elsevier Science B.V., Amsterdam, 2006, pp. 433–492.
[91] I. Muhammad, V. Samoylenko, Expert Opin. Drug Discov. 2 (2007) 1065–1084.
[92] J.C. Prot, J.M. Kornprobst, C R Acad. Sci. Ser. 296 (1983) 555–557.
[93] J.C. Prot, J.M. Kornprobst, Rev. Nematol. 8 (1985) 383–389.
[94] I. Watanabe, K. Koike, T. Satou, T. Nikaido, Biol. Pharm. Bull. 23 (2000) 723–726.
[95] T. Kuriyama, X.-L. Ju, S. Fusazaki, H. Hishinuma, T. Satou, K. Koike, T. Nikaido, Y. Ozoe, Pest. Biochem. Physiol. 81 (2005) 176–187.
[96] E.H. Allen, J. Feldmesser, Phytopathology 60 (1970) 1013.
[97] E.H. Allen, J. Feldmesser, J. Nematol. 3 (1971) 58–61.
[98] S.V. Zinovieva, Z.V. Udalova, I.S. Vasikjeva, V.A. Paseschnichenko, Russ. J. Nematol. 5 (1997) 77–80.
[99] X.-B. Wang, G.-H. Li, L.-J. Zheng, K.-Y. Ji, H. Lu, F.-F. Liu, et al., Chem. Biodivers. 6 (2009) 431–436.
[100] Z.-H. Li, W. Wang, X. Ruan, C.-D. Pan, D.-A. Jiang, Molecules 15 (2010) 8933–8952.
[101] C.D. Mishra, K.C. Mohanty, Indian J. Nematol. 37 (2007) 131–134.
[102] F. Scheffer, R. Kickuth, J.H. Visser, Zeitschrift fuer Pflanzenernaehrung, Duengung. Bodenkunde 98 (1962) 114–120.
[103] D.K. Maheshwari, M. Anwar, J. Phytopathol. 129 (1990) 159–164.
[104] I. Mahmood, Z.A. Siddiqui, Nemtaol. Medit. 21 (1993) 97–98.
[105] R. Mahajan, P. Singh, K.L. Bajaj, Rev. Nematol. 8 (1985) 161–164.
[106] E. Vouyoukalou, E. Stefanoudaki, Nematol. Medit. 26 (1998) 157–160.
[107] N. Sultana, M. Akhter, Z. Khatoon, Nat. Prod. Res. 24 (2010) 407–415.
[108] N. Sultana, M. Akhter, R.A. Khan, N. Afza, R.B. Tareen, A. Malik, Nat. Prod. Res. 24 (2010) 783–788.
[109] A.-L. Molan, A.M. Faraj, Folia Parasitologica 57 (2010) 62–68.
[110] P.H. Evans, W.S. Bowers, E.J. Funk, J. Agric. Food Chem. 32 (1984) 1254–1256.
[111] S. Kawaii, Y. Yoshizawa, J. Mizutani, Biosci. Biotechnol. Biochem. 58 (1994) 982–985.
[112] Y. Yoshizawa, S. Kawaii, M. Kanauchi, M. Chida, J. Mizutani, Biosci. Biotech. Biochem. 57 (1993) 1572–1574.
[113] T.J. Motley, Econ. Bot. 48 (1994) 397–412.
[114] S. Perrett, P.J. Whitfield, Phytother. Res. 9 (1995) 405–409.
[115] P. Morales-Ramirez, E. Madrigal-Bujaidar, J. Mercader-Martinez, M. Cassani, G. Gonzalez, G. Chamorro-Cevallos, M. Salazar-Jacobo, Mutat. Res. Genet. Toxicol. Test 279 (1992) 269–273.
[116] G.-H. Li, L.-Z. Dang, L.-J. Hong, L.-J. Zheng, F.-F. Liu, Y.-J. Liu, K.-Q. Zhang, J. Phytopathol. 157 (2009) 390–392.
[117] T.M. Jurgens, E.G. Frazier, J.M. Schaeffer, T.E. Jones, D.L. Zink, R.P. Borris, J. Nat. Prod. 57 (1994) 230–235.

[118] F. Kiuchi, Y. Goto, N. Sugimoto, N. Akao, K. Kondo, Y. Tsuda, Chem. Pharm. Bull. 41 (1993) 1640–1643.
[119] N.H. Choi, H.R. Kwon, S.W. Son, G.J. Choi, Y.H. Choi, K.S. Jang, S.E. Lee, L.H. Ngoc, J.-C. Kim, Nematology 10 (2008) 801–807.
[120] C.J. Njoku, D.C. Hopp, F. Alali, I.U. Asuzu, J.L. McLaughlin, Planta Med. 63 (1997) 580–581.
[121] G. Burkhardt, H. Becker, M. Grubert, J. Thomas, T. Eicher, Phytochemistry 37 (1994) 1593–1597.
[122] R.A. Momin, R.S. Ramsewak, M.G. Nair, J. Agric. Food Chem. 48 (2000) 3785–3788.
[123] J.A. Gonzalez, A. Estevez-Braun, R. Estevez-Reyes, A.G. Ravelo, J. Chem. Ecol. 20 (1994) 517–524.
[124] C. Goto, S. Kauya, K. Koga, H. Ohtomo, N. Kagei, Parasitol. Res. 76 (1990) 653–656.
[125] S. Valcic, G.A. Wachter, C.M. Eppler, B.N. Timmermann, J. Nat. Prod. 65 (2002) 1270–1273.
[126] N.A. Shakil, J. Pankaj Kumar, R.K. Pandey, D.B. Saxena, Phytochemistry 69 (2008) 759–764.
[127] J.A. Veech, J. Nematol. 14 (1982) 2–9.
[128] R. Hammerschmidt, Annu. Rev. Phytopathol. 37 (1999) 285–306.
[129] M.S.C. Pedras, E.E. Yaya, Phytochemistry 71 (2010) 1191–1197.
[130] J.R. Rich, N.T. Keen, I.J. Thomason, Physiol. Plant Pathol. 10 (1977) 105–106.
[131] D.B. Crane, Rotenone – a new parasiticide. Cornell Veterinarian 23 (1933) 15–31.
[132] M. Stadler, E. Dagne, H. Anke, Planta Med. 60 (1994) 550–552.
[133] R. Cook, S.A. Tiller, K.A. Mizen, R. Edwards, J. Plant Physiol. 146 (1995) 348–354.
[134] R. Edwards, T. Mizen, R. Cook, Nematologica 41 (1995) 51–66.
[135] R.A. Plowright, R.J. Grayer, J.R. Gill, M.L. Rahman, J.B. Harborne, Nematologica 42 (1996) 564–578.
[136] J. Kuc, Annu. Rev. Phytopathol. 33 (1995) 275–297.
[137] D.T. Kaplan, N.T. Keen, J. Nematol. 9 (1977) 274.
[138] D.T. Kaplan, N.T. Keen, I.J. Thomason, J. Nematol. 10 (1978) 291–292.
[139] D.T. Kaplan, N.T. Keen, I.J. Thomason, Physiol. Plant Pathol. 16 (1980) 309–318.
[140] J.S. Huang, K.R. Barker, Plant Physiol. 96 (1991) 1302–1307.
[141] G.D. Baldridge, N.R. O'Neill, D.A. Samac, Plant Mol. Biol. 38 (1998) 999–1010.
[142] X.-B. Wang, G.-H. Li, L. Li, L.-J. Zheng, R. Huang, K.-Q. Zhang, Nat. Prod. Res. 22 (2008) 666–671.
[143] S. Nakajima, K. Takaishi, Y. Nagano, Y. Alen, K. Kawazu, N. Baba, Okayama Daigaku Nogakubu Gakujutsu Hokoku 93 (2004) 1–4.
[144] R.A. Momin, M.G. Nair, J. Agric. Food Chem. 49 (2001) 142–145.
[145] M. Wink, In: T.A. van Beek, H. Breteler (Eds.), Phytochemistry and Agriculture, Oxford Science Publishers, Oxford, 1993, pp. 171–213.
[146] N. Wuyts, R. Swennen, D. De Waele, Nematology 8 (2006) 89–101.
[147] J.D. Bijloo, Nematologica 11 (1965) 643–644.
[148] M.V. Chandravadana, E.S.J. Nidiry, R.M. Khan, M.S. Rao, Fundam. Appl. Nematol. 17 (1994) 185–192.
[149] K. Matsuda, M. Kimura, K. Komai, M. Hamada, Agric. Biol. Chem. 53 (1989) 2287–2288.
[150] K. Matsuda, K. Yamada, M. Kimura, M. Hamada, J. Agric. Food Chem. 29 (1991) 189–191.
[151] B.-G. Zhao, J. Chem. Ecol. 25 (1999) 2205–2214.
[152] M. Onda, K. Takiguchi, M. Hirakura, H. Fukushima, M. Akagawa, F. Naoi, Nippon Nagei-kaguku Kaishi 39 (1965) 168–170.
[153] M. Onda, K. Abe, K. Yonezawa, N. Esumi, T. Suzuki, Chem. Pharm. Bull. 18 (1970) 435–439.
[154] T. Satou, M. Koga, R. Matsuhashi, K. Koike, I. Tada, T. Nikaido, Vet. Parasitol. 104 (2002) 131–138.

[155] G.-X. Wang, Z. Zhou, D.-X. Jiang, J. Han, J.-F. Wang, L.-W. Zhao, J. Li, Vet. Parasitol. 171 (2010) 305–313.
[156] A.N.E. Birch, W.M. Robertson, I.E. Geoghegan, W.J. McGavin, T.J.W. Alphey, L.E. Fellows, A.A. Watson, M.S.J. Simmonds, E.A. Porter, Brighton Crop Protection Conference – Pests and Diseases, 1 (1992) 67–72.
[157] Q. Yu, J.W. Potter, J. Food Agric. Environ. 6 (2008) 428–432.
[158] G. Fassuliotis, G.P. Skucas, J. Nematol. 1 (1969) 287–288.
[159] T.C. Thoden, M. Boppre, J. Hallmann, Nematology 9 (2007) 343–349.
[160] T.C. Thoden, M. Boppre, J. Hallmann, Pest. Manag. Sci. 65 (2009) 823–830.
[161] J. Auman, Int. J. Parasitol. 22 (1992) 87–90.
[162] E.S.J. Nidiry, R.M. Khan, P.P. Reddy, Nematol. Medit. 21 (1993) 127–128.
[163] D.O. Morgan, J. Helminthol. 3 (1925) 185–192.
[164] C. Ellenby, Ann. Appl. Biol. 32 (1945) 67–70.
[165] S.G. Donkin, M.A. Eiteman, P.L. Williams, J. Nematol. 27 (1995) 258–262.
[166] M.J. Potter, K. Daies, A.J. Rathjen, J. Chem. Ecol. 24 (1998) 67–80.
[167] P.J. Islip, In: M.E. Wolff (Ed.), fifth ed., Burger's Medicinal Chemistry and Drug Discovery: Therapeutic Agents, vol. 4, John Wiley & Sons, New York, 1997, pp. 365–413.
[168] C. Ellenby, Ann. Appl. Biol. 38 (1951) 859–875.
[169] E.M. Smedley, J. Helminthol. 17 (1939) 31–38.
[170] A.W. Johnson, J. Feldmesser, In: J.A. Veech, D.W. Dickson (Eds.), Vistas on Nematology, Soc. Nematol., Hyattsville, MD, 1987, pp. 448–454.
[171] M. Mitarai, J.C.V. Sicat, Y. Uchida, Y. Okada, M. Nigata, Bull. Fac. Agric. Miyazaki Univ. 44 (1997) 35–44.
[172] Q. Yu, R. Tsao, M. Chiba, J. Potter, J. Food Agric. Environ. 3 (2005) 218–221.
[173] R. Kermanshai, B.E. McCarry, J. Rosenfeld, P.S. Summers, E.A. Weretilnyk, G.J. Sorger, Phytochemistry 57 (2001) 427–435.
[174] G.N. Jing, J.M. Halbrendt, J. Penn. Acad. Sci. 68 (1994) 29–33.
[175] L. Lazzeri, R. Tacconi, S. Palmieri, J. Agric. Food Chem. 41 (1993) 825–829.
[176] S. Pinto, E. Rosa, S. Santos, Acta Hortic. 459 (1998) 323–327.
[177] J.M. Halbrendt, J. Nematol. 28 (1996) 8–14.
[178] P.D. Brown, M.J. Morra, Adv. Agron. 61 (1997) 167–231.
[179] B.A. Halkier, L. Du, Trends Plant Sci. 2 (1997) 425–431.
[180] I.A. Zasada, H. Ferris, Phytopathology 93 (2003) 747–750.
[181] M.J. Potter, V.A. Vanstone, K.A. Davies, J.A. Kirkegaard, A.J. Rathjen, J. Nematol. 31 (1999) 291–298.
[182] R.W. McLeod, C.C. Steel, Nematology 1 (1999) 613–624.
[183] S. Buskov, B. Serra, E. Rosa, H. Sorensen, J.C. Sorensen, J. Agric. Food Chem. 50 (2002) 690–695.
[184] B. Serra, E. Rosa, R. Iori, J. Barillari, A. Cardoso, C. Abreu, P. Rollin, Sci. Hortic. 92 (2002) 75–81.
[185] L. Lazzeri, G. Curto, O. Leoni, E. Dallavalle, J. Agric. Food Chem. 52 (2004) 6703–6707.
[186] S. Bak, S.M. Paquette, M. Morant, A.V. Morant, S. Saito, N. Bjarnholt, M. Zagrobelny, K. Jørgensen, T. Hamann, S. Osmani, H.T. Simonsen, R.S. Perez, T.B. van Heeswijck, B. Jørgensen, B.L. Moller, Phytochem. Rev. 5 (2006) 309–329.
[187] A.F. Robinson, W.W. Carter, J. Nematol. 18 (1986) 563–570.
[188] D.B. Tattersall, S. Bak, P.R. Jones, C.E. Olsen, J.K. Nielsen, M.L. Hansen, P.B. Hoj, B.L. Moller, Science 293 (2001) 1826–1828.
[189] T.L. Widmer, Plant Dis. 84 (2000) 562–568.

[190] B.A. Halkier, B.L. Moller, Plant Physiol. 96 (1991) 10–17.
[191] K.A. Nielsen, D.B. Tattersall, P.R. Jones, B.L. Moller, Phytochemistry 69 (2008) 88–98.
[192] T.L. Widmer, G.S. Abawi, J. Nematol. 34 (2002) 16–22.
[193] G.W. Butler, B.G. Butler, Nature 187 (1960) 780–781.
[194] J.E. Poulton, Plant Physiol. 94 (1990) 401–405.
[195] C.P. Magalhaes, J. Xavier-Filho, F.A.P. Campos, Phytochem. Anal. 11 (2000) 57–60.
[196] J.J.D. Ponte, B.S. Cavada, J. Silveira-Filho, Fitopathol. Bras. 21 (1996) 489–498.
[197] R. Gupta, N.K. Sharma, Int. J. Pest Manag. 39 (1993) 390–392.
[198] M. Takasugi, Y. Yachida, M. Anetai, T. Masamune, K. Kegasawa, Chem. Lett. 1 (1975) 43–44.
[199] J. Yang, W. Gao, Q. Liu, J. Tianjin Norm. Univ. (Nat. Sci. Ed.) 30 (2010) 69–72.

Chapter 4

Beneficial Health Effects of Bioactive Compounds Present in Spices and Aromatic Herbs

Fernández-López Juana, Pérez-Alvarez José Angel and Viuda-Martos Manuel

IPOA Research Group (UMH-1 and REVIV-Generalitat Valenciana), AgroFood Technology Department, Escuela Politécnica Superior de Orihuela, Miguel Hernández University, Crta. Beniel km 3.2. Orihuela Alicante, Spain.

INTRODUCTION

The use of spices and aromatics herbs was a common practise in ancient times and in the present days. These products impart aroma, colour and taste to food preparations and sometimes mask undesirable odours. Volatile oils give the aroma, and oleoresins impart the taste [1]. The literature describes how they impart flavour and reduce the need for salt and fatty condiments, improve digestion and provide the organism with extra antioxidants that prevent the appearance of physiological and metabolic alterations [2]. Thus, since prehistoric times, herbs have also been the basis for nearly all medicinal therapy until synthetic drugs were developed in the nineteenth century [3]. Prior to the nineteenth century, plant medicines were administered mostly in their crude forms as infusions (herbal teas), tinctures (alcoholic extracts), decoctions (boiled extract of roots or bark), syrups (extracts of herbs made with syrup or honey) or applied externally as ointments (poultices, balms and essential oils (EOs)) and herbal washes [4,5]. However, during the late nineteenth and early twentieth centuries, scientists began isolating, purifying and identifying active ingredients (principles) from plant extracts.

Within the increasing interest to survive a healthy life the use of plants with pharmaceutical properties has received increased interest nowadays from both homeopathic and allopathic ranches. Besides, these medicinal plants play an important role in public health, especially in developing countries [6–8]. According to World Health Organization [9] 60% of the world's population depend on traditional medicine, and 80% of the population in developing countries depend almost entirely on traditional medical practises, in particular,

Bioactive Natural Products (Part Q), Vol. 37. http://dx.doi.org/10.1016/B978-0-444-59514-0.00004-3

herbal medicine for their primary health care needs [10,11]. As for developed countries, it is reported that sufferers of chronic diseases are turning to herbal remedies as alternatives to modern synthetic drugs [12]. This renewed interest in the use of herbal medicine in developed countries is believed to be motivated by several factors including: side effects of modern drugs, the effectiveness of plant remedies and the high cost of synthetic drugs.

In recent years major research has been focused on the biologically active derivatives of medicinal plants for the development of novel potential drugs for several pathologies with significant social impact [13] mainly the potential anti-tumour activity and the protective cardiovascular health. The components of spices responsible for the quality attributes have been designated as active principles and in many instances they are also responsible for the health beneficial physiological effects [14]. These bioactive compounds are defined as non-nutritive constituents of spices or aromatic herbs, which usually occur in very small quantities. A vast diversity of bioactive components in plants has been found: terpenes, lignans, sulfides, carotenoids, cumarins, saponins, phytosterols, and polyphenols, including flavonoids, anthocyanins and phenolic acids [15].

Phenolic compounds and monoterpens are major constituents present in different spices; interestingly, the effective ingredients in several plant-derived medicinal extracts are also flavonoids [16].

A review of literature involving research of medicinal plants suggests that scientists follow more or less the same general strategy to investigate these compounds for their pharmacological properties [17,18]. This general strategy can be summarized in:

a) Selection of plant species,
b) Collection and identification of plant material,
c) Extraction of plant material,
d) Screening the extract for biological activity,
e) Bioassay guided fractionation and isolation of active compounds,
f) Preclinical and clinical studies.

MONOTERPENES

Monoterpenes is the name given to a substantial group of vegetal components with a common biosynthetic origin; they are the fundamental component of spices EOs [19]. The most investigated monoterpenes are eugenol, carvacrol, γ-terpinene, carvone, thymol and limonene (Fig. 1) especially regarding their anticarcinogenesis activity, antioxidant properties and LDL reduction activity.

Monoterpenes and Anticarcenogenesis Activity

In animal models and at cellular levels, a number of dietary monoterpenes show anti-tumour activities preventing the initiation and progression of cancer and also causing regression of existing malignant tumours [16]. Thus, Kim *et al*. [20]

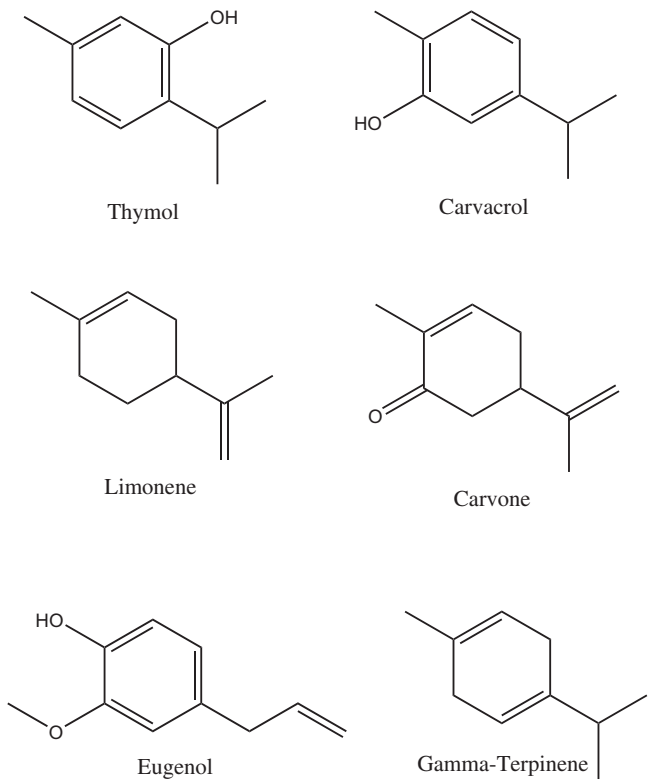


FIGURE 1 Structures of some important monoterpenes present in spices and aromatic herbs.

investigated the effect of eugenol on the growth and cyclooxygenase-2 (COX-2) expression in HT-29 human colon cancer cells. These authors concluded that eugenol inhibited the proliferation of HT-29 cells and the mRNA expression of COX-2, but not COX-1. Yoo *et al*. [21] reported that eugenol transduced the apoptotic signal *via* ROS generation, thereby inducing mitochondrial permeability transition (MPT), reducing anti-apoptotic protein Bcl-2 level, inducing cytochrome *c* release to the cytosol, and subsequent apoptotic cell death in human promyelocytic leukaemia cells (HL-60). Han *et al*. [22] demonstrated that eugenol reduced the levels of dimethylbenz[a]anthracene-induced (DMBA) DNA adduct formation in the breast cancer cell line, MCF-7 through at least three different mechanisms, (i) the direct inhibition and (ii) suppression of DMBA-activating enzymes and (iii) the induction of DMBA detoxification enzymes. Pisano *et al*. [23] reported that dimeric forms (biphenyls) of eugenol elicited specific antiproliferative and pro-apoptotic activity on neuroectodermal tumour cells, possibly indicating their anti-cancer effect. Ghosh *et al*. [24] have shown that eugenol inhibits growth and induces apoptosis in melanoma cells *in vitro* and inhibits invasion and metastasis *in vivo*. Taken together, these

findings underscore the pro-apoptotic, anti-invasive and anti-angiogenic potential of eugenol. In this way Manikandan *et al*. [25] shows that the administration of eugenol induced apoptosis *via* the mitochondrial pathway by modulating the Bcl-2 family proteins, Apaf-1, cytochrome *c*, and caspases and inhibiting invasion, and angiogenesis as evidenced by changes in the activities of metalloproteinases and the expression of matrix metalloproteinase-2 and metalloproteinase-9, vascular endothelial growth factor (VEGF), tissue inhibitors of metalloproteinases-2 and reversion-inducing cysteine rich protein with Kazal motifs. These findings are consistent with apoptosis induction by eugenol in mast cells as well as in human cancer cell lines by up regulating the expression of cytochrome *c*, Apaf-1 and caspase-3 associated with PARP cleavage [26]. Pal *et al*. [27] evaluate the chemopreventive potential of eugenol in an experimental skin carcinogenesis mice model system. These authors reported a reduction in incidence and sizes of skin tumours along with overall increase in survival of mice were seen due to eugenol treatment. Restriction of skin carcinogenesis at the dysplastic stage along with reduced rate of cellular proliferation and increase in apoptosis were evident in eugenol treated skin tumours. In this way, topical application of eugenol also inhibited skin carcinogenesis in mice by reducing proliferation index and increasing apoptotic index [28]. Slameňová *et al*. [29] demonstrated dose-dependent cytotoxic effects of eugenol in malignant cells (human hepatoma cells HepG2 and human colon cells Caco-2) and also in non-malignant human fibroblasts VH10. The reason for the cytotoxicity of eugenol is probably the induction of apoptosis (programmed cell death connected with morphological changes and fragmentation of DNA). Yogalakshmi *et al*. [30] investigated the preventive effect of eugenol, a naturally occurring food flavouring agent on thioacetamide (TA)-induced hepatic injury in rats. These authors reported that eugenol pre-treatment prevented DNA strand break induced by TA. Increased expression of COX-2 gene induced by TA was also abolished by eugenol. Maralhas *et al*. [31] showed that eugenol induces chromosomal aberrations and endo-reduplication in V79 cells, in the absence of an exogenous biotransformation system, suggesting a direct acting genotoxic mechanism, possibly as a topoisomerase II inhibitor. Abraham [32] indicated that eugenol pre-treatment can lead to a moderate reduction in genotoxicity of cyclophosphamide, procarbazine, *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine and urethane.

Arunarsee [33] showed induction of apoptosis by carvacrol in MDA-MB 231 cells dose dependently at an IC50 of 100mM with a decrease in the mitocondrial membrane potential of the cells resulting in release of cytochrome *c* from mitochondria, caspase activation and cleavage of PARP. For this author carvacrol induced morphological changes such as cell shrinkage, rounding of cells and membrane blebbing which depict the induction of apoptosis. This induction of apoptosis appears to be mediated by cell cycle arrest at S phase, increase in Annexin V positive cells, decrease in mitochondrial membrane potential and increase in cytochrome *c* release from mitochondria, decrease in Bcl-2/Bax ratio, increase in caspase activity and cleavage of PARP and fragmentation of

DNA. Thus, the current work clearly indicates that carvacrol could be a potent anti-tumour molecule against metastatic breast cancer cells. Carvacrol inhibited DMBA-induced tumourigenesis in rats and the growth of melanomas *in vitro* [34]. Ipek *et al*. [35] found that carvacrol prevented mutagenicity of known mutagens in lymphocytes and inhibited the DNA synthesis in mouse myoblasts. Koparal and Zeitinoglu [36] reported that carvacrol applied A549 cell lines, increase in dose of carvacrol caused a decrease in cell number, degeneration of cell morphology and a decrease in total protein amount. Cells were treated with carvacrol were seen to have detached from the disk, with cell rounding, cytoplasmic blebbing and irregularity in shape. In a study Mehdi *et al*. [37], showed the dose-dependent cytotoxic effect of carvacrol in human cervical cancer HeLa and SiHa cells at an IC50 of 50 mg L^{-1} by both the cytotoxic assays, respectively. The dying cells showed characteristics of apoptosis such as DNA fragmentation. Zeytinoglu *et al*. [38] informed that the incubation of the cells with different doses of carvacrol prevented DNA synthesis in the growth medium and ras-activating medium, which contains dexamethasone. This result demonstrates that carvacrol inhibits growth of myoblast cells even after activation of mutated *N-ras* oncogene. Karkabounas *et al*. [39] indicated that carvacrol exerts *in vivo* remarkable anticarcinogenic effects. This is supported by: (a) 30% lower tumour incidence in Wistar rats when the carcinogen was incubated in carvacrol in comparison with the tumour incidence by 3,4 benzopurene not incubated in carvacrol; (b) the significant prolongation of survival time of animals treated with 3,4 benzopurene in the presence of carvacrol compared with the control group; (c) the significant decrease of 3,4 benzopirene carcinogenic potency (44%), and (d) the induction of tumours of lower malignancy in the experimental group compared with the control group.

Limonene prevents both mammary and liver cancers at the promotion/progression stage. When mammary cancers were initiated in rats by either the direct acting carcinogen *N*-methyl-*N*-nitrosourea or the indirectly acting carcinogen DMBA, they could be prevented from developing if the carcinogen-exposed rats were fed limonene starting 2 weeks after carcinogen doping [40]. Haag *et al*. [41] tested the ability of limonene to induce the regression of chemically induced small mammary carcinomas, which occasionally spontaneously regress. They found that a large but nontoxic dose of limonene could cause the complete regression of the majority of treated mammary tumours, they also observed that advanced (>10mm diameter) chemically induced mammary tumours, which only rarely spontaneously regress. Russin *et al*. [42] reported that *d*-limonene diets as low as 0.1 and 0.01% were effective in significantly increasing tumour latency in rats with DMBA-induced mammary cancer. Chen *et al*. [43] evaluated the antiproliferative effect in human prostate cancer LNCaP cells. L-limonene was weak cell growth inhibitor and introduction of 4-(2-methoxyphenyl) piperazine to limonene significantly increased their antiproliferative effect. The antiproliferative effect was correlated with ERK activation and $p21^{waf1}$ induction. Uedo *et al*. [44] reported that long-term oral administration of 2%

limonene significantly decreased the incidence of gastric cancers. Limonene also significantly decreased the labelling index and significantly increased the apoptotic index of gastric cancers. No K-ras mutations were detected in gastric cancers induced by MNNG in either group. These findings indicate that limonene inhibits the development of gastric cancers through increased apoptosis and decreased DNA synthesis of gastric cancers, but not through *ras* oncoprotein plasma membrane association. Crowell *et al*. [45] examined the effect on 26 limonene-like monoterpenes on protein isoprenylation and found that limonene inhibits protein isoprenylation and that many monoterpenes inhibited small G protein isoprenylation to a greater extent than limonene did.

Archana *et al*. [46] demonstrated that thymol especially at a concentration of 25μg/ml has the potential of protecting cells from radiation-induced apoptosis. However, the exact mechanism of inhibition of radiation-induced apoptotic pathway by TOH needs to be elucidated. Aydin *et al*. [47] who noticed that non-cytotoxic low concentrations of thymol, protected against DNA strand breakage, induced by semiquinone and oxygen radicals formed by 2-amino-3-methylimidazo(4,5-f)-quinoline (IQ) and mitomycin C (MMC) in lymphocytes, whereas high concentrations increased DNA damage.

d-Carvone has been found to reduce fore stomach tumour formation and pulmonary adenoma formation induced by *N*-nitrosodiethylamine in mice [48]. Yu *et al*. [49] reported that *d*-carvone shows antiproliferative effects and induced apoptosis in human HL-60 cells through an ROS-independent pathway that is different from the treatment with As_2O_3 and doxorubicin.

Monoterpenes and Cardiovascular Health

One of the major risk factors for the development of coronary heart disease is dyslipidemia, which is mainly characterized by elevated levels of low-density lipoprotein cholesterol (LDL-C) and/or reduced high-density lipoprotein cholesterol (HDL-C) [50]. There are scientific evidences that free radical oxidation of the low-density lipoproteins (LDLs) plays an important role in the development of atheroma plaque [51]. Thus, Milde *et al*. [52] informed that incubating plasma with γ-terpinene extends the resulting lag phase in the copper-mediated formation of conjugated dienes in LDL by the factor 1.5 or 2.7 respectively results, corroborated by Graßmann *et al*. [53] who proved that γ-terpinene can be enriched in LDL by pre-incubating human blood plasma with γ-terpinene and that the subsequently isolated LDL shows a high resistance against copper induced oxidation. Takahashi *et al*. [54] showed in later studies that γ-terpinene, which was added to LDL solutions is able to prevent the oxidation of LDL. Carvacrol and thymol, *via* post-transcriptional actions, suppress 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase activity, the rate limiting for the synthesis of cholesterol [55]. Inhibition of this enzyme in the liver stimulates LDL receptors, leading to increased clearance of LDL from the bloodstream and a decrease in blood cholesterol levels [56].

PHENOLICS ACIDS

One of the main compounds responsible for most of the functional properties of many foods, among them herbs and spices, are phenolic acids in any of their forms [19]. The structures of phenolic acids are diverse (Fig. 2). Hydroxybenzoic acids are based on a C6–C1 skeleton, while cinnamic acids are a series of *trans*-phenyl-3-propenoic acids with C6–C3 structures differing in their ring substitution. Caffeic acid (CA) and its esters and ferulic acid are the most frequently encountered phenolic acids in plant foods [57].

Almost all phenolics possess several common biological and chemical properties: (a) antioxidant activity, (b) the ability to scavenge electrophiles, (c) the ability to inhibit nitrosation, (d) the ability to chelate metals, (e) the potential to producing hydrogen peroxide in the presence of certain metals, and (f) the capability to modulate certain cellular enzyme activities [58]. All these characteristics make phenolic acids to be considered as bioactive compounds with beneficial health properties.

Anticarcenogenesis Activities of Spices Phenolic Acids

A wide array of phenolic substances, particularly those present in dietary and medicinal plants, has been reported to possess substantial anticarcinogenic and antimutagenic activities. The majority of these naturally occurring phenolics possess anti-oxidative and anti-inflammatory properties, which appear to

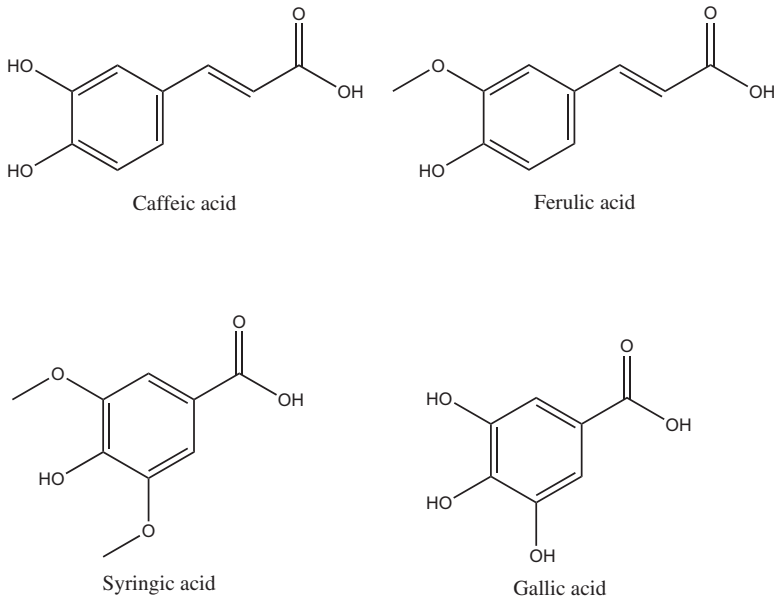


FIGURE 2 Some examples of phenolic acids present in spices and aromatic herbs.

contribute to their chemopreventive or chemoprotective activity. Kampa *et al*. [59] demonstrated the antiproliferative and apoptotic effects of selective phenolic acids on T47D human breast cancer cells. They suggested that all six phenolic acids tested (CA, ferulic acid, protocatechuic acid, 3,4-dihdroxyphenylacetic acid, sinapic acid and syringic acid) showed a dose-dependent and time-dependent inhibitory antiproliferative effect on T47D cells, with the following potency: CA > ferulic acid = protocatechuic acid = 3,4-dihdroxy-phenylacetic acid > sinapic acid = syringic acid.

Caffeic acid (3,4-dihydroxycinnamic acid) as mentioned above, is found in plant foods. Jung *et al*. [60] examined *in vitro* and *in vivo*, whether CA exerts anti-cancer activity by targeting signal transducer and activator of transcription 3 (STAT3). They found that CA significantly inhibits STAT3 activity, and that this in turn down-regulates HIF-1a activity. Consequently, sequential blockade of STAT3 and HIF-1a resulted in the down-regulation of VEGF by inhibiting their recruitment to the VEGF promoter. In mice bearing a Caki-I carcinoma, CA retarded tumour growth and suppressed STAT3 phosphorylation, HIF-1a expression, vascularization and STAT3-inducible VEGF gene expression in tumours. Chang *et al*. [61] reported that CA significantly reduced proliferation of cervical cancer cells (HeLa) in a concentration-dependent manner. Morphological evidence of apoptosis, including nuclei fragmentation was clearly observed 24 and 48h after exposure to CA (1mM and 10mM). Indeed, CA decreased levels of uncleaved caspase-3 and Bcl-2, and induced cleaved caspase-3 and p53. For these authors CA induces apoptosis by inhibiting Bcl-2 activity, leading to release of cytochrome *c* and subsequent activation of caspase-3, indicating that CA induces apoptosis *via* the mitochondrial apoptotic pathway. Kim *et al*. [62] demonstrated that CA has the anti-angiogenic activity to retinal endothelial cells and retinal neovascularization in a mouse model, which might be related to the suppression of ROS-induced VEGF expression. CA effectively inhibited VEGF-induced proliferation of retinal endothelial cells in concentration-dependent manner. In addition, VEGF-induced migration and tube formation of retinal endothelial cells were completely inhibited. This anti-angiogenic activity of CA on retinal endothelial cells was related to the antioxidant activity: the inhibitory activity of CA to H2O2-induced reactive oxygen species (ROS) production and VEGF expression.

Another phenolic acid with anticarcinogenesis activity is the gallic acid (3,4,5-triphydroxyl-benzoic acid). Gallic acid is a well-known natural antioxidant which is reported to induce apoptosis in various cell lines such as PLC/PRF/5 (human hepatoma), HL-60 RG (human promyelocytic leukaemia), P-388 D1 (mouse lymphoid neoplasma), HeLa (human epithelial carcinoma), dRLh-84 (rat hepatoma) and KB (human epidermoid carcinoma) but not in human T-cell leukaemia (MOLT-4) and erythroleukemic (K-562) cell lines through generation of ROS, Ca2+ influx and activation of calmodulin [63]. Apoptosis induced by gallic acid is associated with oxidative stresses derived from ROS,

mitochondrial dysfunction and an increase in intracellular Ca2+ level [64,65]. However, gallic acid shows no cytotoxicity against normal fibroblast and endothelial cells [66]. You and Park [67] investigated the anti-cancer effects of gallic acid on Calu-6 and A549 lung cancer cells in relation to ROS and glutathione (GSH). Gallic acid dose dependently decreased the growth of Calu-6 and A549 cells with an IC50 of approximately 10–50μM and 100–200μM gallic acid at 24h, respectively. Gallic acid also induced cell death in lung cancer cells, which was accompanied by the loss of mitochondrial membrane potential. Ho *et al*. [68] investigated the effect gallic acid on the inhibition of gastric adenocarcinoma (AGS). These authors informed that the expression of MMP-2/9 of AGS cells was inhibited by 2.0μM of gallic acid. It is possible that the suppressive effect of gallic acid on MMP-2/9 might involve the inhibition of NF-jB activity. Multiple proteins involved in metastasis and the cytoskeletal reorganization signal pathway, including Ras, Cdc42, Rac1, RhoA, RhoB, PI3K and p38MAPK, were also inhibited by gallic acid.

Ferulic acid (4-hydroxy-3-methoxy cinnamic acid) is an ubiquitous phenolic compound of plant tissues and thus constitutes a bioactive ingredient of many foods. Baskaran *et al*. [69] investigated the chemopreventive potential of ferulic acid on DMBA-induced mammary carcinogenesis in Sprague–Dawley rats. The authors reported that oral administration of ferulic acid at a dose of 40mg/kg body weight to rats treated with DMBA significantly prevented the tumour formation in 80% of animals. Also, oral administration of ferulic acid significantly protected the biochemical and molecular abnormalities in DMBA-treated rats. Although the exact mechanism for the chemopreventive potential of ferulic acid in DMBA-induced mammary carcinogenesis is unclear, its antigenotoxic and antioxidant potential as well as modulatory effect on phase II detoxification cascade could play a possible role. In a similar study, Alias *et al*. [70] evaluated and compared the chemopreventive potential of topically applied and orally administered ferulic acid in DMBA-induced skin carcinogenesis. These authors showed that the oral administration of ferulic acid completely prevented the formation of skin tumours, whereas topically applied ferulic acid did not show significant chemopreventive activity during DMBA-induced mouse skin carcinogenesis. Also, oral administration of ferulic acid reverted the status of phase I and phase II detoxication agents, lipid peroxidation by-products and antioxidants to near-normal range in DMBA-treated mice. This is probably due to its modulating effect on the status of lipid peroxidation, antioxidants and detoxication agents during DMBA-induced skin carcinogenesis.

Antioxidant Activities of Spices Phenolic Acids

Many herbal spices are known as excellent sources of natural antioxidants, and consumption of fresh herbs in the diet may therefore contribute to the daily antioxidant intake. Phenolic acids are natural antioxidants and most of

their pharmacological properties are considered to be due to their antioxidant action. It is considered that the antioxidant activity of phenolic compounds is due to their high redox potentials, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers [71]. The antioxidant activity of the phenolics is essentially determined by their structures, in particular the electron delocalization over an aromatic nucleus. When these compounds react with free radicals, the delocalization of the gained electron over the phenolic antioxidant occurs, and the stabilization by the resonance effect of the aromatic nucleus, which prevents the continuation of the free radical chain reaction [72]. Earlier studies on antioxidant effect of caffeic and ferulic acid pertain to radical scavenging, inhibition of lipid peroxidation and protection against LDL oxidation [73,74]. Caffeic acid and its derivatives are good substrates of polyphenol oxidases, and under certain conditions may undergo oxidation in plant tissues or products of plant origin [75]. This compound has been shown to be a α-tocopherol protectant in LDL [76]. Production of ROS in tissue contributes to the development of various chronic diseases such as cancer, neurodegenerative diseases, and cardiovascular diseases [77]; it is well known that ROS are responsible for ischemia/reperfusion (I/R). Thus, Sato *et al*. [78] found that chlorogenic acid and CA exhibit protective effects against I/R injury in the rat small intestine. However, uptake of chlorogenic acid by Caco-2 cells was much less than that of CA, which is a metabolite of chlorogenic acid. Since chlorogenic acid is hydrolyzed into CA in the intestine and CA has a stronger antioxidant activity than that of chlorogenic acid, it is possible that CA plays an important role in the protective effect of chlorogenic acid against I/R injury.

Ferulic acid has been shown to possess some activity towards peroxynitrite [79] and oxidized low-density lipoprotein (ox-LDL) *in vitro* [80]. The antioxidant action of ferulic in plasma is far greater than that of vitamin C against LDL oxidation [81]. In a similar study Itagaki *et al*. [82] reported that combined antioxidant activity from radical scavenging and xanthine oxidase inhibition of ferulic acid was much weaker than the combined antioxidant activities of (−)-epigallocatechin gallate and ascorbic acid, treatment with ferulic acid also prevented the increase in vascular permeability caused by intestinal I/R. It is possible that chain-breaking activity plays a contributory role in the protective effect of ferulic acid on oxidative injury in humans and *in vivo* studies.

FLAVONOIDS

Flavonoids are ubiquitous polyphenolic compounds comprised of several classes including flavonols, flavanones, flavanols, flavones and catechins [83]. Fig. 3 shows some examples of flavonoids. Many studies have pointed to the functional properties of flavonoids, which include anti-inflammatory [84] capacities; their cardioprotective [85] and anticarcinogenic [86] activities.

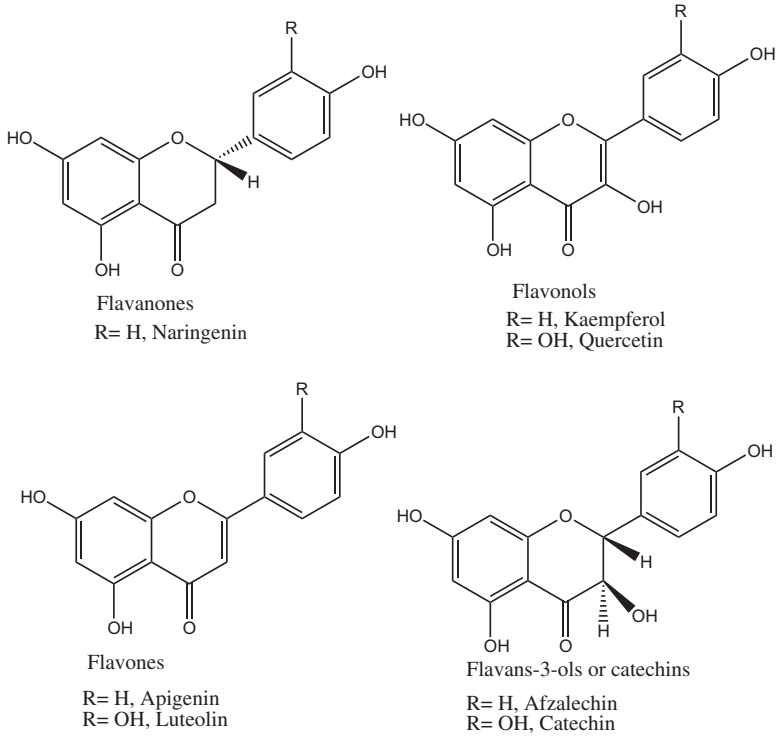


FIGURE 3 Some examples of flavonoids present in spices and aromatic herbs.

Anticarcenogenesis Activities of Spices Flavonoids

Rosmarinic acid (α-*O*-caffeoyl-3,4-dihydroxyphenyl lactic acid) is a naturally occurring hydroxylated compound [87] and it is found in Lamiaceae family. Huang *et al*. [88], informed that rosmarinic acid inhibited several important steps of angiogenesis including proliferation, migration, adhesion and tube formation of human umbilical vein endothelial cells (HUVEC) in a concentration-dependent manner. Rosmarinic acid at the concentration of 12.5, 25, 50, 100 and 200mM reduced cell growth by 6.2, 8.0, 13.0, 15.4 and 24.0%. Rosmarinic acid also reduced intracellular ROS level, H_2O_2-dependent VEGF expression and IL-8 release of endothelial cells. These findings suggested that the anti-angiogenic potential of RA might be related to its anti-oxidative activity, which further resulted in the inhibition of ROS associated VEGF expression and IL-8 release. Kim *et al*. [89] investigated the inhibitory effect of rosmarinic acid on ADR-induced apoptosis in H9c2 cardiac muscle cells. These authors showed that rosmarinic acid has a greater anti-apoptotic effect than probucol, ascorbic acid and alpha-tocopherol. The results provide evidence for the first time that c-Jun N-terminal (JNK) kinase and signal-regulated kinase (ERK) activation can be pro-apoptotic in ADR-treated cardiac muscle cells, and strongly suggest

that ROS is responsible for ADR-induced JNK, and ERK activation. In addition, these results also suggest that the inhibitory effect of RA is associated with the up-regulation of Mn-SOD and glutatione and its scavenging effect on free radicals in cardiac muscle cells. Furtado *et al*. [87] evaluated the mutagenic and/or antimutagenic potential of rosmarinic acid on peripheral blood cells of Swiss mice. These authors reported that treatment with different concentrations of rosmarinic acid combined with doxorubicin revealed a significant reduction in the frequency of micronuclei compared to animals treated with doxorubicin only. Although the exact mechanism underlying the protective effect of RA is not completely understood, the putative antioxidant activity of this compound might explain its effect on DXR mutagenicity.

Kaempferol (3',4',5,7-tetrahydroxyflavone) and quercetin (3,3',4',5,7-pentahydroxyflavone) are flavonols, commonly found in spices and aromatic herbs. Reported that quercetin aglycone has been shown to interact with some receptors, particularly an aryl hydrocarbon receptor, which is involved in the development of cancers induced by certain chemicals. Murakami *et al*. [90] reported that quercetin aglycone has also been shown to modulate several signal transduction pathways involving MEK/ERK and Nrf2/keap1, which are associated with the processes of inflammation and carcinogenesis. Yuan *et al*. [91] reported that the intravenous injection (50mg/kg) of a liposomal preparation of quercetin efficiently suppressed tumour growth in mice models transplanted with diverse cancer cells. Oh *et al*. [92] informed that quercetin inhibits VEGF mediated angiogenesis in TAM-resistant breast cancer cells and that the suppression of PI3-kinase-dependent Pin1 expression by quercetin seems to be critical for this activity.

Taking kaempferol into account, Yoshida *et al*. [93] informed that the combined treatment with kaempferol and factor-related apoptosis-inducing ligand (TRAIL) drastically induced apoptosis in human colon cancerSW480 cells, compared to single treatments. Kaempferol markedly up-regulated TRAIL receptors, DR5and DR4. DR5 but not DR4 siRNA efficiently blocked apoptosis induced by the co-treatment with kaempferol and TRAIL, indicating that DR5 up-regulation by kaempferol helps to enhance TRAIL actions. Zhang *et al*. [94] studied the effect and mechanisms of kaempferol on pancreatic cancer cell proliferation and apoptosis. These authors reported that upon the treatment with 70μm kaempferol for 4 days, MIA PaCa-2 cell proliferation was significantly inhibited by 79% compared with control cells. Similarly, the treatment with kaempferol significantly inhibited Panc-1 cell proliferation. Kaempferol treatment also significantly reduced ^{3}H-thymidine incorporation in both MIA PaCa-2 and Panc-1 cells.

Apigenin (4',5,7-trihydroxy flavone) is found in high amounts in several herbs. Lee *et al*. [95] investigated whether combination therapy with gemcitabine and apigenin enhanced anti-tumour efficacy in pancreatic cancer. The authors reported that *in vitro*, the combination treatment resulted in more growth inhibition and apoptosis through the down-regulation of NF-jB activity

with suppression of Akt activation in pancreatic cancer cell lines (MiaPaca-2, AsPC-1). *In vivo*, the combination therapy augmented tumour growth inhibition through the down-regulation of NF-jB activity with the suppression of Akt in tumour tissue. The combination of gemcitabine and apigenin enhanced anti-tumour efficacy through Akt and NF-jB activity suppression and apoptosis induction. Zhong *et al*. [96] found that pro-apoptotic proteins (NAG-1 and p53) and cell cycle inhibitor (p21) were induced in the presence of apigenin, and kinase pathways including PKCd and ataxia telangiectasia mutated (ATM), play an important role in activating these proteins. Indeed, apigenin is able to reduce polyp numbers, accompanied by increasing p53 activation through phosphorylation in animal models. Shukla and Gupta [97] conducted detailed studies to understand its mechanism of action. The exposure of human prostate cancer 22Rv1cells, harbouring wild-type p53, to growth-suppressive concentrations (10–80μM) of apigenin resulted in the stabilization of p53 by phosphorylation on critical serine sites, p14ARF-mediated down-regulation of MDM2 protein, inhibition of NF-κB/p65 transcriptional activity, and induction of p21/WAF-1 in a dose- and time-dependent manner.

Cardioprotective Activities of Spices Flavonoids

The beneficial effects derived from flavonoids, with respect to cardiovascular disease prevention have been attributed to (i) their antioxidant activity [98], (ii) the prevention of atherosclerosis [99], and (iii) the effect on platelet aggregation [100]. Many studies focused on the protective effects of flavan-3-ols such as catechin against lipid peroxidation and LDLs oxidation. Thus Auclair *et al*. [101] investigated the anti-atherosclerotic effects of catechin supplemented in the diet of apoE deficient mice at a low nutritional level. They reported that catechin supplementation reduced the mean atherosclerotic lesion area by 32% but had no effect on total cholesterol and triacylglycerol levels in the plasma and the liver. Indeed, the expression of 450 genes was significantly modified by catechin supplementation. Some of the most significantly down-regulated genes included genes coding for adhesion molecules such as CD34 and PSGL-1 known to play a key role in leukocyte adhesion to the endothelium. Koo and Noh [102] has determined, in *in vitro* and *in vivo* studies, that catechins inhibit the intestinal absorption of dietary lipids. Studies *in vitro* indicated that catechins interfere with the emulsification, digestion, and micellar solubilization of lipids, critical steps involved in the intestinal absorption of dietary fat, cholesterol, and other lipids. Based on the observations, it was likely that catechins lower the absorption and tissue accumulation of other lipophilic organic compounds. Mangiapane *et al*. [103] reported that catechin completely inhibited the copper catalyzed oxidation of LDL at 20mg/l.

Bhaskar *et al*. [104] reported that supplementation of quercetin significantly modulates the NF-κB p65 nuclear translocation. The cytokine IL-6 production was significantly increased in ox-LDL treated group and was decreased by

quercetin treatment. Quercetin also decreased the mRNA expression of inducible enzymes like COX-2 and iNOS. Indeed Quercetin inhibited the ox-LDL induced toll-like receptors 2 and toll-like receptors 4 expression at mRNA level and modulated the Toll-like receptors-NF-κB signalling pathway thereby inhibited the cytokine production and down regulated the activity of inflammatory enzymes thus have protective effect against the ox-LDL induced inflammation in PBMCs. Gong *et al*. [105] explored the roles of quercetin in the regulation of Paraoxonase (1 PON1) expression, serum and liver activity and protective capacity of HDL against LDL oxidation in rats. Group feeding quercetin (10mg/L) increased (a) hepatic expression of PON1 by 35%, (b) serum and liver PON1 activities by 29% and 57%, respectively, and (c) serum homocysteine thiolactonase (HCTL) activity by 23. These results suggest that quercetin has anti-atherogenic effect by up regulating PON1 gene expression and its protective capacity against LDL oxidation.

Anti-inflammatory Activities of Spices Flavonoids

The topical application of spices and extracts is common practise for alleviating a variety of discomforts such as backache, rheumatism, skin rashes and inflammatory processes in general [106]. Mahat *et al*. [107] investigate whether kaempferol modulates the cyclooxygenase pathway *via* inhibition of nitric oxide production, which in turn contributes to its anti-inflammatory activity. In other study Pang *et al*. [108] informed that kaempferol at 5, 10, and 20μM inhibited the TNF-α-induced production of IL-6 in mouse osteoblasts in a dose-dependent manner. García-Mediavilla *et al*. [109] informed that kaempferol produced a significant concentration-dependent decrease of iNOS, COX-2 and CRP protein level at all concentrations assayed. Indeed, the kaempferol significantly inhibited mRNA level of iNOS, COX-2, and CRP. For these authors the modulation of iNOS, COX-2 and CRP by kaempferol may contribute to the anti-inflammatory effects of these two flavonoids in Chang Liver cells, *via* mechanisms likely to involve blockade of NF-κB activation and the resultant up-regulation of the pro-inflammatory genes.

Quercetin is another flavonoid with demonstrated anti-inflammatory activity. Studies carried out *in vitro* have shown that quercetin exhibits, besides other activities, pronounced anti-inflammatory property, an effect that seems to be associated with its ability to block some inflammatory mediators [110], adhesion molecules expression [111], inducible enzymes [109] and nuclear transcription factor activation [112]. Thus, quercetin blocked both the cyclooxygenase and lipooxygenase pathways at relatively high concentrations; while at lower concentrations, the lipooxygenase pathway was the primary target of inhibitory anti-inflammatory activity [113]. In this way quercetin attenuated the expression of pro-inflammatory cytokines in PMA and calcium ionophore (PMACI)-stimulated mast cells and suppressed the TNF-α induced recruitment of NF-kappa B to pro-inflammatory gene promoters in

murine epithelial intestinal cells [114,115]. Rogerio *et al*. [116] reported that oral administration of quercetin suspension failed to interfere with leukocyte recruitment, while quercetin-loaded microemulsion inhibited in a dose-dependent way, the eosinophil recruitment to the broncho alveolar lavage fluid quercetin-loaded microemulsion also significantly reduced both IL-5 and IL-4 levels, but failed to interfere with CCL11, IFN-γ and LTB4 levels. In addition, quercetin-loaded microemulsion oral treatment inhibited the nuclear transcription factor kappa B (NF-κB) activation, P-selectin expression and the mucus production in the lung.

Funakoshi-Tago *et al*. [117] found that apigenin, luteolin and fisetin significantly inhibited TNFα-induced NF-κB transcriptional activation; however, they had no effect on the degradation of IκB. proteins and the nuclear translocation and DNA binding activity of NF-κB p65. Interestingly, the suppression of NF-κB activation by these flavonoids is due to inhibition of the transcriptional activation of NF-κB, since the compounds markedly inhibited the transcriptional activity of GAL4-NF-κB p65 fusion protein. In another study Deqiu *et al*. [118] demonstrated that in cells pre-treated with luteolin, IKKb phosphorylation were reduced, which, in turn, blocked the NF-kB activation through attenuating P65 phosphorylation. At the same time, it was also found that the gene over-expressions for TNF-α and IL-6 were also reduced by luteolin pre-treatment. A recent study showed that apigenin flavonoid reduced the production of inflammatory mediators by inhibiting NF-κB activation; however, the molecular mechanism of how apigenin inhibits NF-κB activation and consequentially exhibits anti-inflammatory activity has not been described [119]. Poeckel *et al*. [120] described the anti-inflammatory activity of carnosic acid and carnosol, compounds of a phenolic nature occurring in spices such as rosemary and sage, which act through the formation of pro-inflammatory compounds such as leukotrienes.

CONCLUSIONS

This review shows the beneficial effects of different bioactive compounds, such as terpenoids, flavonoids and phenolics acids present in spices and aromatic herbs and how they can help in the fight against certain diseases. However, one must keep in mind that most studies are performed with isolated bioactive components *in vitro* or *in vivo* assays (cell lines or animals studies), which means that the concentration used is higher than that is present in the spices or aromatic herbs. So, several studies must be developed, especially in issues such as bioavailability and bioaccessibility, to determine how the spices or aromatic herbs consumed in a balanced diet can help to maintain a proper health. We must also take into account that their use as nutraceuticals in a concentrated form needs further investigation for efficacy and toxicity. On the other hand, we must also take the possible synergistic effect produced by various bioactive compounds in food these adjuncts.

REFERENCES

[1] V.A. Parthasarathy, B. Chempakam, T.J. Zachariah, In: V.A. Parthasarathy, B. Chempakam, T.J. Zachariah (Eds.), Chemistry of Spices, CAB Int, Wallingford, 2008, pp. 1–20.

[2] J.A. Pérez-Alvarez, J. Fernandez-Lopez, E. Sayas-Barberá, In: J.A. Perez-Alvarez, E. Sayas-Barberá, J. Fernandez-Lopez (Eds.), NonEnFundamentos Tecnológicos y Nutritivos de la Dieta Mediterranea, Universidad Miguel Hernandez, Elche, 2002, pp. 103–119.

[3] S. Dragland, H. Senoo, K. Wake, K. Holte, R. Blomhoff, J. Nutr. 133 (2003) 1286–1290.

[4] B. Griggs, Green Pharmacy, A History of Herbal Medicine. J.Norman & Hobhouse Ltd., London, 1981.

[5] A. Gurib-Fakim, Mol. Aspects Med. 27 (2006) 1–93.

[6] M. Viuda-Martos, E. Sendra, J.A. Perez-Alvarez, J. Fernandez-Lopez, M. Amensour, J. Abrini, J. Essent. Oil Res. 23 (2011) 1–9.

[7] A. Ocaña-Fuentes, E. Arranz-Gutiérrez, F.J. Señorans, G. Reglero, Food Chem. Toxicol. 48 (2010) 1568–1575.

[8] M. Viuda-Martos, A.E.G.S. El Gendy, E., Sendra, J. Fernández-López, K.A.A. El Razik, E.A. Omer, J.A. Pérez-Alvarez, J. Agric. Food. Chem. 58 (2010) 9063–9070.

[9] WHO, Legal Status of Traditional Medicines and Complementary/Alternative Medicine: A Worldwide Review. vol. 1WHO Publishing, , 2001.

[10] N.R. Fransworth, Ethnobotany and the search for new drugs. In: D.J. Chadwick, J. Marsh (Eds.), CIBA Foundation Symposium 185, John Wiley and Sons, Chichester, New York, 1994, pp. 42–51.

[11] B.B. Zhang, D.E. Moller, Curr. Opin. Chem. Biol. 4 (2000) 461–467.

[12] J.B. Calixto, Braz. J. Med. Biol. Res. 33 (2) (2000) 179–189.

[13] M. Heinrich, S. Gibbons, J. Pharmacol. 53 (2001) 425–432.

[14] K. Srinivasan, K. Sambaiah, N. Chandrasekhara, Food Rev. Int. 2 (2004) 187–220.

[15] E. Capecka, A. Mareczek, M. Leja, Food Chem. 93 (2005) 223–226.

[16] K.H. Wagner, I. Elmadfa, Ann. Nutr. Metab. 47 (2003) 95–106.

[17] M. Heinrich, J. Barnes, S. Gibbons, E.M. Williamson, Fundamentals of Pharmacognosy and Phytotherapy. Churchill Livingstone, Elsevier Science Ltd., UK, 2004.

[18] A.D. Kinghorn, M.F. Balandrin, Human Medical Agents from Plants. American Chemical Society, San Francisco, USA, 1993.

[19] M. Viuda-Martos, Y. Ruiz-Navajas, J. Fernández-López, J.A. Pérez-Alvarez, Crit. Rev. Food Sci. Nutr. 51 (1) (2011) 13–28.

[20] S.S. Kim, O.J. Oh, H.Y. Min, E.J. Park, Y. Kim, H.J. Park, Y.N. Han, S.K. Lee, Life Sci. 73 (2003) 337–348.

[21] C.B. Yoo, K.T. Han, K.S. Cho, J. Ha, H.J. Park, J.H. Nam, U.H. Kil, K.T. Lee, Cancer Lett. 225 (2005) 41–52.

[22] E.H. Han, Y.P. Hwang, T.C. Jeong, S.S. Lee, J.G. Shin, H.G. Jeong, FEBS Lett. 581 (2007) 749–756.

[23] M. Pisano, G. Pagnan, M. Loi, M.E. Mura, M.G. Tilocca, G. Palmieri, D. Fabbri, M.A. Dettori, G. Delogu, M. Ponzoni, C. Rozzo, Mol. Cancer 6 (8), 2007.

[24] R. Ghosh, N. Nadiminty, J.E. Fitzpatrick, W.L. Alworth, T.J. Slaga, A.P. Kumar, J. Biol. Chem. 280 (2005) 5812–5819.

[25] P. Manikandan, R. Senthil, R.V. Priyadarsini, G. Vinothini, S. Nagini, Life Sci. 86 (2010) 936–941.

[26] H.K. Park, D.W. Han, Y.H. Park, J.C. Park, Curr. Appl. Phys. 5 (2005) 449–452.

[27] D. Pal, S. Banerjee, S. Mukherjee, A. Roy, C.K. Panda, S. Das, J. Dermatol. Sci. 59 (2010) 31–39.

[28] G. Kaur, M. Athar, M.S. Alam, Mol. Carcinog. 49 (2010) 290–301.
[29] D. Slameňová, E. Horváthová, L. Wsólová, M. Šramková, J. Navarová, Mutat. Res. Genet. Toxicol. Environ. Mutagen. 677 (2009) 46–52.
[30] B. Yogalakshmi, P. Viswanathan, A.C. Venkatraman, Toxicology 68 (3) (2010) 204–212.
[31] A. Maralhas, A. Monteiro, C. Martins, M. Kranendonk, A. Laires, J. Rueff, A.S. Rodrigues, Mutagenesis 21 (2006) 199–204.
[32] S.K. Abraham, Food Chem. Toxicol. 39 (5) (2001) 493–498.
[33] K.M. Arunasree, Phytomedicine 17 (2010) 581–588.
[34] L. He, H. Mo, S. Hadisusilu, A.A. Quresni, C.E. Elson, Biochem. Mol. J. Nutr. 127 (1997) 668–674.
[35] E. Ipek, B.A. Tuylu, H. Zeytinoglu, Cytotechnology 43 (2004) 145–148.
[36] A.T. Koparal, M. Zeytinoglu, Cytotechnology 43 (2003) 149–154.
[37] S.J. Mehdi, A. Ahmad, M. Irshad, N. Manzoor, M.M.A. Rizvi, Biol. Med. 3 (2) (2001) 307–312.
[38] H. Zeytinoglu, Z. Incesu, K.H.C. Baser, Phytomedicine 10 (4) (2003) 292–299.
[39] S. Karkabounas, O.K. Kostoula, T. Daskalou, P. Veltsistas, M. Karamouzis, I. Zelovitis, A. Metsios, P. Lekkas, A.M. Evangelou, N. Kotsis, I. Skoufos, Exp. Oncol. 28 (2) (2006) 121–125.
[40] L.W. Wattenberg, Cancer Res. 43 (1983) 2448–2453.
[41] J.D. Haag, M.J. Lindstrom, M.N. Gould, Cancer Res. 52 (1992) 4021–4026.
[42] W.A. Russin, J.D. Hoesly, C.E. Elson, M.A. Tanner, M.N. Gould, Carcinogenesis 10 (1989) 2161–2164.
[43] J. Chen, M. Lu, Y. Jing, J. Dong, Bioorg. Med. Chem. 14 (19) (2006) 6539–6547.
[44] N. Uedo, M. Tatsuta, H. Iishi, M. Baba, N. Sakai, H. Yano, T. Otani, Cancer Lett. 137 (2) (1999) 131–136.
[45] P.L. Crowell, Z. Ren, S. Lin, E. Vedejs, M.N. Gould, Biochem. Pharmacol. 47 (1994) 1405–1415.
[46] P.R. Archana, B.N. Rao, M. Ballal, B.S. Rao, Mutat. Res. Genet. Toxicol. Environ. Mutagen. 680 (2009) 70–77.
[47] S. Aydin, A.A. Basaran, N. Basaran, Mutat. Res. 581 (2005) 43–53.
[48] L.W. Wattenberg, V.L. Sparnins, G. Barany, Cancer Res. 49 (10) (1989) 2689–2692.
[49] Z. Yu, W. Wang, L. Xu, J. Dong, Y. Ping, Asian J. Trad. Med. 3 (4) (2008) 134–143.
[50] A. Esmaillzadeh, L. Azadbakht, J. Nutr. 138 (8) (2008) 1469–1475.
[51] C.P. Cannon, Clin. Cornerstone 8 (6) (2007) 14–23.
[52] J. Milde, E.F. Elstner, J. Graßmann, Phytomedicine 11 (2, 3) (2004) 105–113.
[53] J. Graßmann, D. Schneider, D. Weiser, E.F. Elstner, Forsch/Drug Res. 51 (2001) 799–805.
[54] Y. Takahashi, N. Inaba, S. Kuwahara, W. Kuki, Biosci. Biotechnol. Biochem. 67 (1) (2003) 195–197.
[55] C.E. Elson, J. Nutr. 125 (1995) 1666–1672.
[56] E.K. Akkol, G. Avcı, I. Küçükkurt, H. Keleş, U. Tamer, S. Ince, E. Yesilada, J. Ethnopharmacol. 126 (2) (2009) 314–319.
[57] F.A. Tomas-Barberan, M.N. Clifford, J. Sci. Food Agric. 80 (2000) 1024–1032.
[58] F. Shahidi, C.T. Ho, Phenolic compounds in foods and natural health products. In: F. Shahidi (Ed.), ACS Symposium Series, American Chemical Society, Washington, DC, 2005, pp. 1–8.
[59] M. Kampa, V.I. Alexaki, G. Notas, A.P. Nifli, A. Nistikaki, A. Hatzoglou, E. Bakogeorgou, E. Kouimtzoglou, G. Blekas, D. Boskou, A. Gravanis, E. Castanas, Breast Cancer Res. 6 (2004) 63–74.

[60] J.E. Jung, H.S. Kim, C.S. Lee, D.H. Park, Y.N. Kim, M.J. Lee, J.W. Lee, J.W. Park, M.S. Kim, S.K. Ye, M.H. Chung, Carcinogenesis 28 (8) (2007) 1780–1787.
[61] W.C. Chang, C.H. Hsieh, M.W. Hsiao, W.C. Lin, Y.C. Hung, J.C. Ye, Taiwan J. Obstet. Gynecol. 49 (4) (2010) 419–424.
[62] J.H. Kim, B.J. Lee, J.H. Kim, Y.S. Yu, K.W. Kim, Vasc. Pharmacol. 51 (4) (2009) 262–267.
[63] S. Qin, P.B. Chock, Biochemistry 40 (2001) 8085–8091.
[64] H.M. Chen, Y.C. Wu, Y.C. Chia, F.R. Chang, H.K. Hsu, Y.C. Hsieh, C.C. Chen, S.S. Yuan, Cancer Lett. 286 (2009) 161–171.
[65] M. Inoue, N. Sakaguchi, K. Isuzugawa, H. Tani, Y. Ogihara, Biol. Pharm. Bull. 23 (2000) 1153–1157.
[66] M. Inoue, R. Suzuki, N. Sakaguchi, Z. Li, T. Takeda, Y. Ogihara, B.Y. Jiang, Y. Chen, Biol. Pharm. Bull. 18 (1995) 1526–1530.
[67] B.R. You, W.H. Park, Toxicol. In Vitro 24 (2010) 1356–1362.
[68] H.H. Ho, C.S. Chang, W.C. Ho, S.Y. Liao, C.H. Wu, C.J. Wang, Food Chem. Toxicol. 48 (9) (2010) 2508–2516.
[69] N. Baskaran, S. Manoharan, S. Balakrishnan, P. Pugalendhi, Eur. J. Pharmacol. 637 (2010) 22–29.
[70] L.M. Alias, S. Manoharan, L. Vellaichamy, S. Balakrishnan, C.R. Ramachandran, Exp. Toxicol. Pathol. 61 (2009) 205–214.
[71] M.G. Miguel, Flav. Fragr. J. 25 (2010) 291–312.
[72] R. Tsao, Z. Deng, J. Chromatogr. B 812 (2004) 85.
[73] J.C. Cheng, F. Dai, B. Zhou, L. Yang, Z.L. Liu, Food Chem. 104 (2007) 132–139.
[74] W.M. Wu, L. Lu, Y. Long, T. Wang, L. Liu, Q. Chen, R. Wang, Food Chem. 105 (2007) 107–115.
[75] D. Bassil, D.P. Makris, P. Kefalas, Food Res. Int. 38 (2005) 395–402.
[76] J. Laranjinha, O. Vieira, V. Madeira, L. Almeida, Arch. Biochem. Biophys. 323 (1995) 373–381.
[77] I.F. Benzie, Eur. J. Nutr. 39 (2000) 53–61.
[78] Y. Sato, S. Itagaki, T. Kurokawa, J. Ogura, M. Kobayashi, T. Hirano, M. Sugawara, K. Iseki, Int. J. Pharm. 403 (2011) 136–138.
[79] R. Pannala, B. Razaq, S. Halliwell, C.A. Rice-Evans, Free Rad. Biol. Med. 24 (1998) 594–606.
[80] H. Schroeter, R.J. Williams, R. Matin, L. Iversen, C.A. Rice-Evans, Free Rad. Biol. Med. 29 (2000) 1222–1233.
[81] G.P. Castelluccio, C. Bolwell, C. Gerrish, C.A. Rice-Evans, Biochem. J. 316 (1996) 691–694.
[82] S. Itagaki, T. Kurokawa, C. Nakata, Y. Saito, S. Oikawa, M. Kobayashi, T. Hirano, K. Iseki, Food Chem. 114 (2009) 466–471.
[83] C. Manach, A. Scalbert, C. Morand, C. Remesy, L. Jimenez, Am. J. Clin. Nutr. 79 (2004) 727–747.
[84] J. Lameira, C.N. Alves, L.S. Santos, A.S. Santos, R.H. de Almeida Santos, J. Souza, C.C. Silva, A.B.F. da Silva, J. Mol. Struct. Theochem. 862 (1–3) (2008) 16–20.
[85] J.M. Hodgson, K.D. Croft, Mol. Aspects Med. 31 (6) (2010) 495–502.
[86] V.P. Androutsopoulos, A. Papakyriakou, D. Vourloumis, A.M. Tsatsakis, D.A. Spandidos, Pharmacol. Ther. 126 (2010) 9–20.
[87] M.A. Furtado, L.C. Fernandes de Almeida, R.A. Furtado, W.R. Cunha, D.C. Tavares, Mutat. Res. 657 (2008) 150–154.
[88] S.S. Huang, R.L. Zheng, Cancer Lett. 239 (2006) 271–280.

[89] D.S. Kim, H.R. Kim, E.R. Woo, S.T. Hong, H.J. Chae, S.W. Chae, Biochem. Pharmacol. 70 (2005) 1066–1078.
[90] A. Murakami, H. Ashida, J. Terão, Cancer Lett. 269 (2008) 315–325.
[91] Z.P. Yuan, L.J. Chen, L.Y. Fan, M.H. Tang, G.L. Yang, H.S. Yang, X.B. Du, G.Q. Wang, W.X. Yao, Q.M. Zhao, B. Ye, R. Wang, P. Diao, W. Zhang, H.B. Wu, X. Zhao, Y.Q. Wei, Clin. Cancer Res. 12 (10) (2006) 3193–3199.
[92] S.J. Oh, O. Kim, J.S. Lee, J.A. Kim, M.R. Kim, H.S. Choi, J.H. Shim, K.W. Kang, Y.C. Kim, Food Chem. Toxicol. 48 (11), 2010. 3227–3224.
[93] T. Yoshida, M. Konishi, M. Horinaka, T. Yasuda, A.E. Goda, H. Taniguchi, K. Yano, M. Wakada, T. Sakai, Biochem. Biophys. Res. Commun. 375 (2008) 129–133.
[94] Y. Zhang, A.Y. Chen, M. Li, C. Chen, O. Yao, J. Surg. Res. 148 (2008) 17–23.
[95] S.H. Lee, J.K. Ryu, K.Y. Lee, S.M. Woo, J.K. Park, J.W. Yoo, Y.T. Kim, Y.B. Yoon, Cancer Lett. 259 (1) (2008) 39–49.
[96] Y. Zhong, C. Krisanapun, S.H. Lee, T. Nualsanit, C. Sams, P. Peungvicha, S.J. Baek, Eur. J. Cancer 46 (18) (2010) 3365–3374.
[97] S. Shukla, S. Gupta, Free Rad. Biol. Med. 44 (10) (2008) 1833–1845.
[98] K.E. Heim, A.R. Tagliaferro, D.J. Bobilya, J. Nutr. Biochem. 13 (2002) 572–584.
[99] E. Tripoli, M. La Guardia, S. Giammanco, D. Di Majo, M. Giammanco, Food Chem. 104 (2007) 466–479.
[100] R.M. Lamuela-Raventos, A.I. Romero-Perez, C. Andres-Lacueva, A. Tornero, Food Sci. Technol. Int. 11 (3) (2005) 159–176.
[101] S. Auclair, D. Milenkovic, C. Besson, S. Chauvet, E. Gueux, C. Morand, A. Mazur, A. Scalbert, Atherosclerosis 204 (2009) 21–27.
[102] S.I. Koo, S.K. Noh, J. Nutr. Biochem. 18 (2007) 179–183.
[103] H. Mangiapane, J. Thomson, A. Salter, S. Brown, G.D. Bell, D.A. White, Biochem. Pharmacol. 43 (1992) 445–450.
[104] S. Bhaskar, V. Shalini, A. Helen, Immunobiology 216 (3) (2011) 367–373.
[105] M. Gong, M. Garige, R. Varatharajalu, P. Marmillot, C. Gottipatti, L.C. Leckey, R.M. Lakshman, Biochem. Biophys. Res. Commun. 379 (2009) 1001–1004.
[106] M.F. Ramadan, Int. J. Food. Sci. Technol. 42 (2007) 1208–1218.
[107] M.Y.A. Mahat, N.M. Kulkarni, S.L. Vishwakarma, F.R. Khan, B.S. Thippeswamy, V. Hebballi, A.A. Adhyapak, V.S. Benade, S.M. Ashfaque, S. Tubachi, B.M. Patil, Eur. J. Pharmacol. 642 (2010) 169–176.
[108] J.L. Pang, D.A. Ricupero, S. Huang, N. Fatma, D.P. Singh, J.R. Romero, N. Chattopadhyay, Biochem. Pharmacol. 71 (2006) 818–826.
[109] V. García-Mediavilla, I. Crespo, P.S. Collado, A. Esteller, S. Sánchez-Campos, M.J. Tuñón, J. González-Gallego, Eur. J. Pharmacol. 557 (2, 3) (2007) 221–229.
[110] S.Y. Cho, S.J. Park, M.J. Kwon, T.S. Jeong, S.H. Bok, W.Y. Choi, Mol. Cell. Biochem. 243 (2003) 153–160.
[111] B. Ying, T. Yang, X. Song, X. Hu, H. Fan, X. Lu, Mol. Biol. Rep. 36 (2009) 1825–1832.
[112] G. Reiterer, M. Toborek, B. Hennig, J. Nutr. 134 (2004) 771–775.
[113] R. Landolfi, R.L. Mower, M. Steiner, Biochem. Pharmacol. 33 (9) (1984) 1525–1530.
[114] P.A. Ruiz, A. Braune, G. Holzlwimmer, L. Quintanilla-Fend, D. Haller, J. Nutr. 137 (2007) 1208–1215.
[115] H.H. Park, S. Lee, H.Y. Son, S.B. Park, M.S. Kim, E.J. Choi, T.S. Singh, J.H. Ha, M.G. Lee, J.E. Kim, M.C. Hyun, T.K. Kwon, Y.H. Kim, S.H. Kim, Arch. Pharm. Res. 31 (2008) 1303–1311.

[116] A.P. Rogerio, C.L. Dora, E.L. Andrade, J.S. Chaves, L.F. Silva, E. Lemos-Senna, J.B. Calixto, Pharm. Res. 61 (4) (2010) 288–297.
[117] M. Funakoshi-Tago, K. Nakamura, K. Tago, T. Mashino, T. Kasahara, Int. Immunopharmacol., 201110.1016/j.intimp.2011.03.012.
[118] Z. Deqiu, L. Kang, Y. Jiali, L. Baolin, L. Gaolin, Biochemistry 93 (3) (2011) 506–512.
[119] L. Xu, L. Zhang, A.M. Bertucci, R.M. Pope, S.K. Datta, Immunol. Lett. 121 (2008) 74–83.
[120] D. Poeckel, C. Greiner, M. Verhoff, O. Rau, L. Tausch, C. Hörnig, D. Steinhilber, M. Schubert-Zsilavecz, O. Werz, Biochem. Pharmacol. 76 (1) (2008) 91–97.

Chapter 5

Bioactive Plant Isoprenoids: Biosynthetic and Biotechnological Approaches

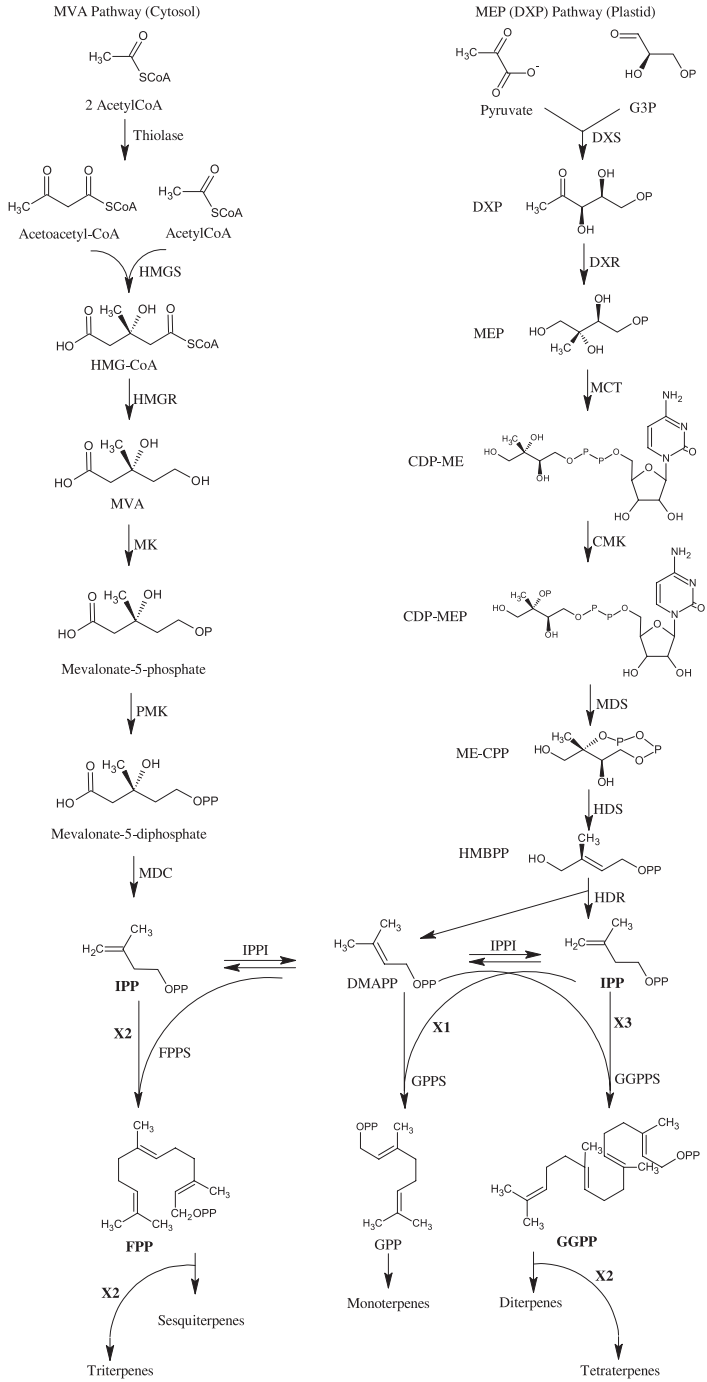
Mariana Galata and Soheil Mahmoud
University of British Columbia, Okanagan Campus, Biology, SCI Building, 3333 University Way, Kelowna, Canada

OVERVIEW OF TERPENOIDS

The isoprenoids – or terpenoids – encompass over 40,000 structurally and functionally diverse natural products. Although present in all living organisms, they are most abundant in plants, where they perform essential functions in growth and development (e.g., as plant growth regulators) and crucial ecological roles (e.g., in defence and pollinator attraction). All isoprenoids are produced through repetitive condensations of the five-carbon unit, isopentyl diphosphate (IPP) and its reactive allylic isomer, dimethyl allyl diphosphate (DMAPP) [1], Fig. 1. There are seven major classes of terpenoids, categorized according to the number of isoprene units that make up their backbone structure [2,3]. These include the mono- (C_{10}), sesqui- (C_{15}), di- (C_{20}), sester- (C_{25}), tri-(C_{30}), tetra- (C_{40}), and polyterpenes (C_n).

Biosynthesis of isoprenoids occurs in four general stages. First is the synthesis of IPP and DMAPP *via* the MVA (mevalonate) and MEP (methylerythritol-4-phosphate) pathways, which are located in separate compartments within a plant cell [4,5], Fig. 1. In plants, the MEP pathway is located in the plastid and through this pathway mono-, di-, and tetraterpenes are produced [5,6]. The MVA pathway is located in the cytosol, where it is responsible for the production of sesqui-, tri-, and polyterpenes [5,7]. Although these two pathways are kept apart and can function independently, there is metabolic exchange between the cytosol and plastid [8]. Secondly, there occurs the formation of the prenyl diphosphates [5,9]. During this stage, one molecule of each of DMAPP and IPP are condensed to produce geranyl diphosphate (GPP) [1,3], the linear precursor to monoterpenes. Two IPPs and one DMAPP are condensed to create farnesyl diphosphate (FPP), the precursor for sesqui- and triterpenes. Three IPPs and one DMAPP are condensed to form geranyl geranyl diphosphate (GGPP), the

Bioactive Natural Products (Part Q), Vol. 37. http://dx.doi.org/10.1016/B978-0-444-59514-0.00005-5


MVA Pathway (Cytosol)
2 AcetylCoA
Thiolase
Acetoacetyl-CoA
AcetylCoA
HMGS
HMG-CoA
HMGR
MVA
MK
Mevalonate-5-phosphate
PMK
Mevalonate-5-diphosphate
MDC
IPP
IPPI
X2
FPPS
FPP
X2
Sesquiterpenes
Triterpenes
MEP (DXP) Pathway (Plastid)
Pyruvate
G3P
DXS
DXP
DXR
MEP
MCT
CDP-ME
CMK
CDP-MEP
MDS
ME-CPP
HDS
HMBPP
HDR
DMAPP
IPPI
IPP
X1
X3
GPPS
GGPPS
GPP
GGPP
Monoterpenes
Diterpenes
X2
Tetraterpenes

precursor for di- and tetraterpenes [9], Fig. 1. The third step involves the synthesis of the parent skeletons for each terpenoid class from the respective prenyl diphosphates (GPP, FPP, and GGPP), *via* terpene synthases and cyclases [3,8]. Finally, there is modification of the terpene parental skeletons, which include electrophilic addition of side groups or rearrangement of the molecule [1,3].

Terpenoids may be synthesized ubiquitously in all plant parts, including leaves, flowers, stems, roots, seeds, and buds. Because terpenoids are toxic to plants, especially in concentrated amounts, those which accumulate large quantities of terpenoids as essential oils or defensive resins, employ specialized structures such as glandular trichomes or resin ducts for storage of these compounds. In this way the toxic terpenoids are sequestered in certain locations of the plants, rather than free-floating throughout their vascular systems [10].

Although once considered secondary, and thus non-essential metabolic by-products, terpenoids perform a variety of crucial functions and have roles in the regulation of growth and development, pigmentation, pollinator attraction, and plant defence. For example, the isoprenoid-derived plant hormones, gibberellins, abscisic acid (ABA), and phytosterols are essential for the growth and development of all the plants [1]. In addition, numerous proteins, polysaccharides, and other key cellular metabolites (e.g., chlorophylls, and quinones) contain isoprenoid (prenyl) side-chains that are essential for their biological activities.

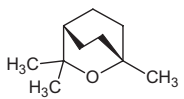
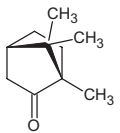
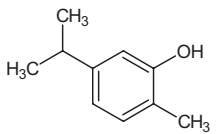
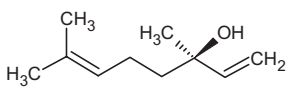
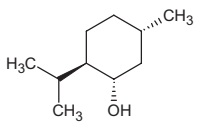
A family of isoprenoids, the carotenoids, create the pigmentation of brightly coloured fruits and some flowers [5,11], and play key roles in attracting pollinating insects by visual means. For example β-carotene produces the orange colour in carrots [11], and the tetraterpenoid lycopene is responsible for the red and orange colours in tomato fruit, Table 1 [12]. Other smaller isoprenoids such as the monoterpenes, linalool and camphene, Table 1, are chemical attractants of insects and other pollinators [9].

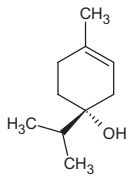
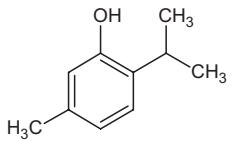
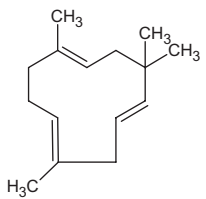
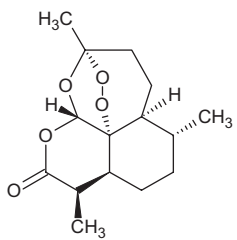
Many isoprenoids have potent antimicrobial properties and play important defensive roles. Notable examples include citral (monoterpene), citronellal (monoterpene) [13], α-cedrene (sesquiterpene), α-duprezianene (sesquiterpene) [14], candidissiol (diterpene), and horminone (diterpene) [15]. Some plant terpenoids are involved in indirect defence, manifested in the form of tritrophic level interactions. An example of a tritrophic system can be seen in the *Phaseolus lunatus*, Lima bean plants. *Tetranychus urticae* are a species of herbivorous spider mites that prey on the Lima beans. These mites are preyed upon by the predatory spider mites, *Phytoseiulus persimilis*. In order to protect themselves from the herbivorous mites, the bean plants produce a cocktail of mono- and sesquiterpenes which attracts the predatory mites [16,17].

In addition to their natural functions, several isoprenoids have been of great medicinal value to humans for centuries. A number of examples are listed in Table 1.

FIGURE 1 Overview of MVA (mevalonate) and MEP (methylerythritol-4-phosphate)/DXP (deoxyxylulose-5-phosphate) pathways of terpenoid biosynthesis in plants. X1 indicates 1 of that molecule: X2 indicates 2 of that molecule, and X3 indicates 3 of that molecule.

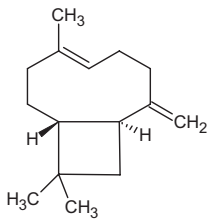
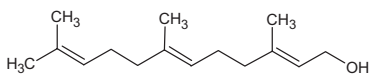
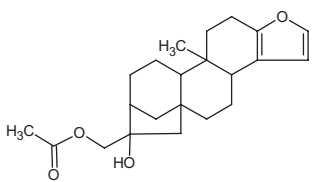
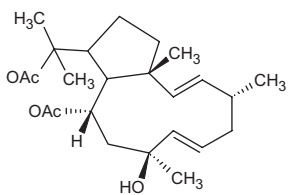
TABLE 1 Representative Examples of Various Classes of Bioactive Plant Terpenoids

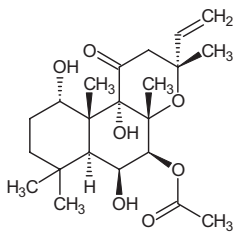
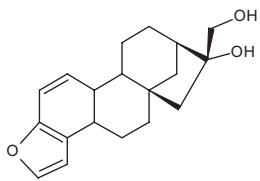
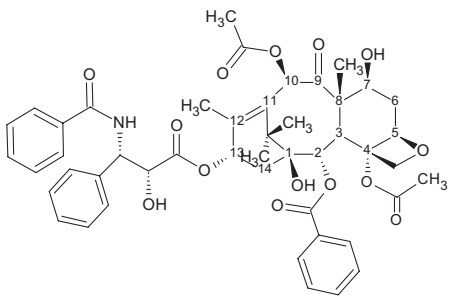
Terpene name	Structure	Class	Medicinal use	Reference
1,8-Cineole		Monoterpene	Decongestant Anti-tussive Local analgesic Anti-inflammatory	[18] [18] [18] [18]
(–)-Camphor		Monoterpene	Topical analgesic	[19]
Carvacrol		Monoterpene	Respiratory disorder	[20]
(–)-Linalool		Monoterpene	Analgesic Anti-inflammatory Anxiolytic	[21] [21] [22]
(+)-Menthol		Monoterpene	Cold symptoms Gastrointestinal disorders Respiratory disorders Muscle pain	[23] [23] [23] [23]

(–)-Terpinen-4-ol		Monoterpene	Anti-cancer (i.e., Melanoma)	[24]
Thymol		Monoterpene	Antioxidant Anti-inflammatory Anti-bacterial (mouth/throat infections) Anti-fungal	[25] [25] [25] [25]
α-Humulene		Sesquiterpene	Anti-inflammatory	[26]
Artemisinin		Sesquiterpene	Malaria Schistosomiasis Hepatitis B Anti-cancer (i.e., Breast)	[27,28] [28] [28] [28]

Continued

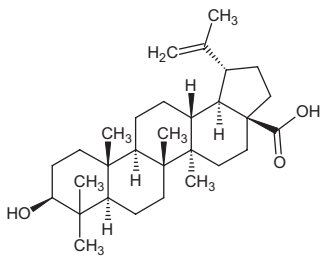
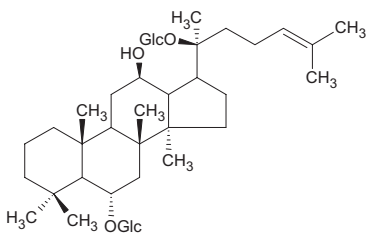
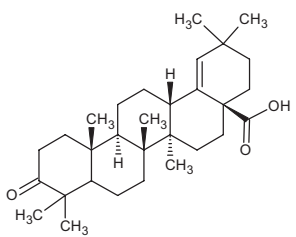
TABLE 1 Representative Examples of Various Classes of Bioactive Plant Terpenoids—Cont'd

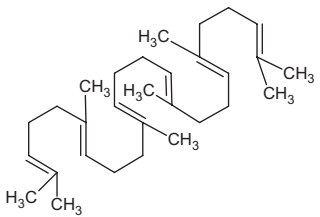
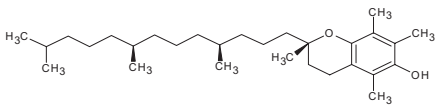
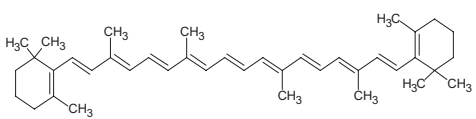
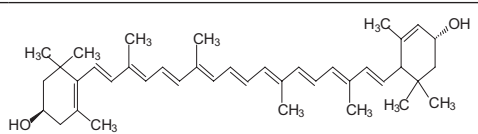
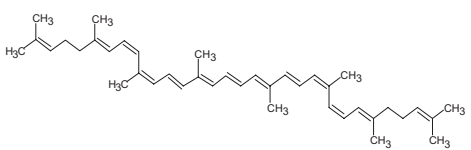
Terpene name	Structure	Class	Medicinal use	Reference
β-Caryophyllene		Sesquiterpene	Anti-inflammatory	[26,29]
Farnesol		Sesquiterpene	Anti-cancer (i.e., Leukemia, lung)	[30]
Cafestol		Diterpene	Anti-cancer (i.e., Hepatic) Skin care (i.e., Psoriasis) Anti-inflammatory	[31,32] [32] [32]
Dolabellane-1		Diterpene	Anti-viral (i.e., HSV-1)	[33,34]

Forskolin		Diterpene	Cardiomyopathy	[35]
Kahweol		Diterpene	Anti-cancer (i.e., hepatic) Skin care (i.e., psoriasis) Anti-inflammatory	[31,32] [32] [32]
Taxol		Diterpene	Cancer (i.e., breast) Anti-diabetic Diuretic Emmenagogue	[36] [37] [37] [37]

Continued

TABLE 1 Representative Examples of Various Classes of Bioactive Plant Terpenoids—Cont'd

Terpene name	Structure	Class	Medicinal use	Reference
Betulinic acid		Triterpene	Anti-viral (i.e., HIV-1) Anti-cancer (i.e., melanoma)	[38] [39]
Ginsenoside (Rg_1)		Triterpene	Adaptogenic agent Diabetes Hypertension Arthritis	[40] [41,42] [41] [43]
Moronic acid		Triterpene	Anti-viral (i.e., HIV-1)	[38]

Squalene		Triterpene	Anti-cancer (i.e., colon and lung)	[44]
(+)-α-Tocopherol		Tetraterpene	Antioxidant Hypertension	[45] [46]
β-Carotene		Tetraterpene	Antioxidant (i.e., macular degeneration)	[47]
(all-E)-Lutein		Tetraterpene	Antioxidant (i.e., macular degeneration)	[48]
Lycopene		Tetraterpene	Antioxidant Anti-cancer (i.e., prostate)	[49] [50]

In recent years there has been increased interest in identification and characterization of the genes involved in the biosynthetic pathways of terpenoids, with the ultimate goal of developing technologies for improving their production in plants [8]. This review will cover the structure, source, biosynthesis, and biotechnological approaches to improve the yields of three individual bioactive plant terpenoids, menthol, artemisinin, and taxol, and two bioactive plant terpenoid classes, ginsenosides and carotenoids (specifically β-carotene).

MENTHOL (MONOTERPENE)

Menthol is a 10-carbon monocyclic terpene alcohol with a molecular weight of 156 and the chemical formula, $C_{10}H_{20}O$, that is naturally produced in plants belonging to the *Mentha* genus in the *Lamiaceae* family [51,52]. One of these is the peppermint plant, *Mentha piperita*, a hybrid cross between *Mentha aquatica* (watermint) and *Mentha spicata* (spearmint) [52]. These perennial herbs were originally found growing in Mediterranean Europe, but are today being cultivated in many different parts of the world [52]. Menthol is responsible for the typical mint odour and taste of these plants and their essential oils [53]. In fact, (−)-menthol, shown in Fig. 2, is the primary and characteristic monoterpene in *Mentha* essential oils and its IUPAC designation is (1*R*,3*R*,4*S*)-1-methyl-4-(1-methylethyl)-cyclohexan-3-ol. In addition to (−)-menthol, there are seven other possible menthol stereoisomers produced by some *Mentha* species: (+)-menthol, (+/−)-isomenthol, (+/−)-neomenthol, and (+/−)-neoisomenthol. These compounds can be obtained from the leaves and stems of these *Mentha* plants (not the roots) *via* steam distillation just before flowering [54,55].

Biological Activities of Menthol

Today, menthol is used in a great variety of products. Some of these include flavouring food, beverages, and candy, fragrance in hygiene products, laundry detergents, air fresheners, pesticides, and pharmaceuticals [55]. As a medicine, menthol is used to treat cold symptoms, gastrointestinal and respiratory disorders, and muscle pain [23]. Examples of over the counter drugs which contain menthol can be seen in Table 2. Menthol is also used as a natural radioprotective agent in cancer patients receiving irradiation therapy. The exact mechanism by which this occurs is yet to be elucidated [56].

It has been shown that a number of mono- and bicyclic terpenoids can affect transient receptor potential vanilloid-3 (TRPV-3) [19]. The TRPV-3′s are part of a super family of ion channels that play roles in the transmission of thermal and nociceptive information in the peripheral nerves, found in human skin. These channels are implicated during situations where peripheral nerves are damaged such as cases of hyperalgesia or inflammation. Among the monocyclic terpenoids tested was (−)-menthol. It was found that after long-term exposure to this monoterpene, TRPV-3 became decreasingly responsive to menthol, indicating

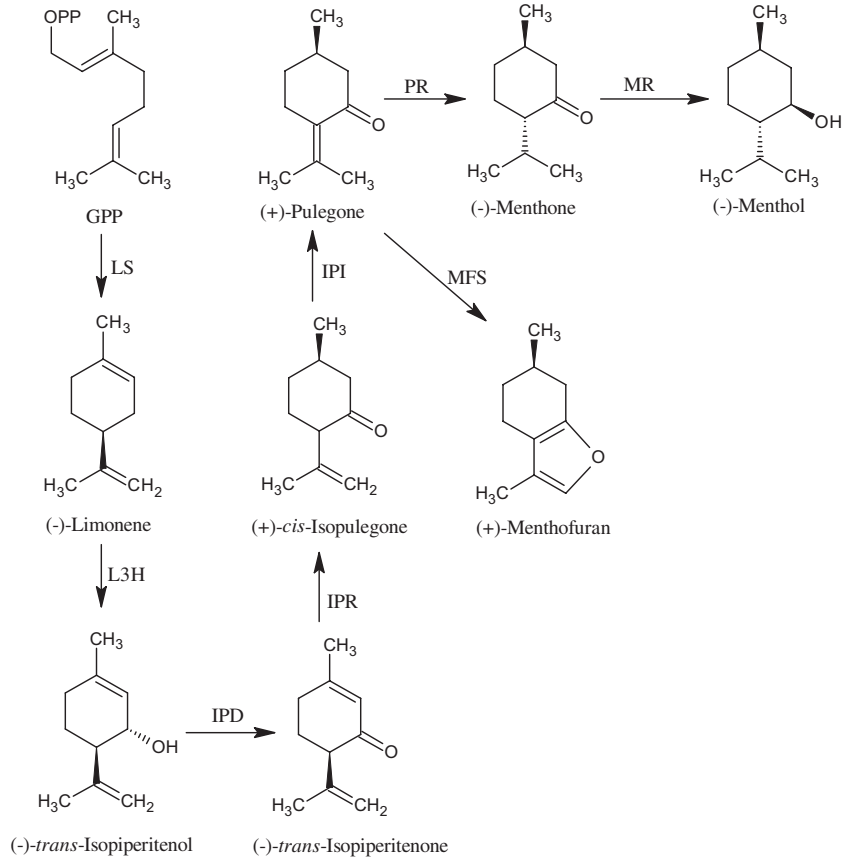


FIGURE 2 The biosynthetic pathway of (−)-menthol in *Mentha* species.

that menthol exerts at least some of its therapeutic properties through desensitization of TRPV-3 [19]. Further, a more recent study has indicated that menthol "cools" through the actions of the TRPM8 (transient receptor potential cation channel subfamily M member 8) ion channel [57]. TRPM8 is part of the same super family of transient receptor potential excitatory ion channels as TRPV-3. TRPM8 cation channels are activated by both menthol and cool temperatures [58,59,60]. Though the receptor on which (−)-menthol acts is now known, the exact mechanism is yet to be elucidated [57].

Biosynthesis of Menthol

The biosynthesis of (−)-menthol is summarized in Fig. 2 [55,61]. Following the condensation of IPP and DMAPP to GPP, the next step is the cyclization of GPP to (−)-limonene, by limonene synthase (LS) [55], which represents the committed and rate-limiting step of menthol biosynthesis [62]. The next step

TABLE 2 Some Examples of Over the Counter Drugs Containing Menthol

Trade name	Use	Vector
IcyHot	Analgesic (topical)	Patch
Tiger balm	Analgesic (topical)	Cream
Ricqlès	Antiemetic	Beverage
Eucerin	Antipuritic	Lotion
Umcka ColdCare	Cough suppressant	Syrup
Mentholatum	Decongestant	Syrup
Vicks Vaporub	Decongestant	Cream
Dermal Em	Sunburn relief	Spray

is carried out by the NADPH dependent limonene-3-hydroxylase (L3H). This enzyme performs the allylic hydroxylation of (−)-limonene, converting it to (−)-*trans*-isopiperitenol [55]. From this point, the third step, an NAD dependent (−)-*trans*-isopiperitenol dehydrogenase (IPD), catalyzes the allylic oxidation of (−)-*trans*-isopiperitenol to produce (−)-isopiperitenone [63]. Next, the NADPH dependent (−)-isopiperitenone reductase (IPR) reduces (−)-isopiperitenone to (+)-*cis*-isopulegone, Fig. 2. The fifth step is the conversion of (+)-*cis*-isopulegone to (+)-pulegone, catalyzed by (+)-*cis*-isopulegone isomerase (IPI) [55]. In the sixth step, PR produces (−)-menthone and (+)-isomenthone. Finally, the seventh step involves the reduction of the carbonyl group on (−)-menthone to produce (−)-menthol. This reduction process is catalyzed by the enzyme (−)-menthone reductase (MR). It has been shown that there are in fact two distinct keto reductases that operate in a stereoselective manner. The first is stereoselective for 3*R* reduction, and yields (−)-menthol from (−)-menthone and (+)-neoisomenthol from (+)-isomenthone. The second is stereoselective for 3*S* reduction and yields (+)-neomenthol from (−)-menthone and (+)-isomenthol from (+)-isomenthone. Of these four isomers produced by the menthol biosynthetic pathway, (−)-menthol is the major product [55,64].

Use of Biotechnology for Improved Production of Menthol

Peppermint, *M. piperita* plant is the main source of menthol for industrial use, and thus biotechnological efforts for improving menthol production have largely focused on improving the quality and yield of the essential oil in this plant. There have been several different methods developed by which this can be achieved, and some of those include: metabolic engineering, somaclones, mutation induction, and fungal endophytes.

In one of the earliest attempts at metabolic engineering in *Mentha* plants, the overexpression of 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) was found to result in an essential oil yield increase of 40–50% in *M. piperita* [65,66]. Similarly, oil yield was increased when geranyl diphosphate synthase (GPPS) was overexpressed, though this modification has yet to be confirmed by field testing [65]. There have been several efforts for overexpression of the LS and L3H genes in peppermint, but this has not resulted in significant increases in yield [67]. In a study by Mahmoud and Croteau, 2004, overexpression of limonene-3-hydroxylase (L3H) resulted in a successful increase in L3H transcript levels in some mint plants, although this did not improve oil yield or quality. However, silencing of the L3H gene led to a substantial increase in the limonene content in the essential oil of these plants, while simultaneously resulting in a much decreased menthol yield [62]. This was due to the lack of a functional L3H enzyme available to continue along the biosynthetic pathway, from (−)-limonene to (−)-menthol. Thus, (−)-menthol biosynthesis was blocked at (−)-limonene, resulting in accumulation of the latter compound [62].

Another biotechnological strategy to improve the quality of menthol production in peppermint involved reducing the levels of pulegone and the menthofuran side products. Menthofuran is considered a "stress metabolite" because it is produced *via* menthofuran synthase (MFS) from (+)-pulegone during stressful environmental conditions such as short sunlight exposure, limited moisture, and nutrient deficiencies [55]. Together, menthofuran and (+)-pulegone give what has been described as an "off" odour to the *Mentha* essential oils. Therefore, a greater quality essential oil would contain lower levels of these metabolites [65]. This was achieved in an experiment by Mahmoud *et al.,* 2003, where there was downregulation of the MFS gene. This led to lower (+)-pulegone and menthofuran levels in the oil, and thus there was an increase in flux through PR, ultimately resulting in greater (−)-menthol content [68].

All the genes of the menthol biosynthetic pathway have been isolated, cloned, and characterized in the recent years and some of those have been successfully manipulated to increase quality and yield of *Mentha* essential oils. However, there are still several enzymes including, IPD, IPR, IPI, PR, and MR, which need to be explored for their metabolic engineering potential, Fig. 2.

As mentioned before, there are other methods, apart from genetic engineering, which are currently used to increase (−)-menthol production. One such technique was developed after the observation that members of the *Mentha* species tend to exhibit highly variable levels of (−)-menthol accumulation between individual plants. Based on this, the ideal means to obtain plants with higher yielding menthol levels would be to establish a breeding program. Unfortunately, *Mentha* plants present the problem of sterility; therefore traditional breeding techniques cannot be used [69]. It has been long known that high monoterpene levels are cytotoxic to plants. Because of this, plants which produce monoterpenes must either store them in specialized structures such

as glandular trichomes or as non-toxic glycosidic derivatives [70]. Using this knowledge, one study showed there was a correlation between *Mentha* plants which could tolerate higher levels of exogenous menthol and those which tended to accumulate more of the monoterpene in their glandular trichomes. It was suggested that the reason for this was due to a natural genetic alteration which afforded the (−)-menthol tolerant plants a mechanism with which to by-pass the feedback inhibition system, while simultaneously allowing for the continued accumulation of very high levels of (−)-menthol (when compared to other *Mentha* plants) [71].

Later, researchers used this observation to help overcome the *Mentha* sterility problem. They turned to natural variant clone selection in order to isolate high yielding clones. An experiment by Dhawan *et al.,* 2003, developed a method for screening somaclones using media containing varying concentrations of menthol to select for high (−)-menthol yielding *Mentha arvensis* plants [70].

An example of the use of mutation induction to create improved *Mentha* lines can be seen in a study which used various clones, hybrids, and fertile lines of the *Mentha* species, and treated them with cobalt 60 gamma irradiation. They found that those plants which had been subjected to irradiation ended up possessing mutations which gave them varying degrees of resistance to *Verticillium dahliae*, a soil borne pathogen, responsible for causing Verticillium wilt in *Mentha* species, whereas untreated control plants remained susceptible to the pathogen [72].

Another interesting method which has been used to increase biosynthesis of (−)-menthol in *Mentha* plants involves endophytic fungi. In 2003, Mucciarelli *et al.* took advantage of the natural mutualistic relationship which can sometimes be found between beneficial fungal endophytes and certain host plants. In these relationships, the endophytic fungus will protect the host plant from harmful fungi. In the case of this experiment, the fungal isolate used was dubbed Plant Growth Promoter-Hyaline Sterile Fungus (PGP-HSF), from the *Pyrenomycetes* class of fungi. *Mentha piperita* plants were inoculated with PGP-HSF and allowed to grow for 30 days, at which point it was found that those plants which had been inoculated with the fungal endophyte had produced up to 65% more (−)-menthol than the control (non-inoculated) plants [73].

ARTEMISININ (SESQUITERPENE)

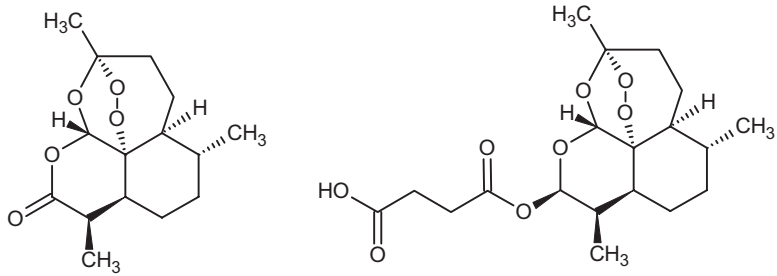
Artemisinin, or qinghaosu, which means “from green herb” in Chinese [74], is produced by plants of the genus *Artemesia*. Some species within this genus include, *Artemesia apiacea* and *Artemesia lancea*, but the species which produces the greatest artemisinin yield is *Artemesia annua*, also known as Qinghao [75] or annual wormwood, a plant which has been used for over 2000 years by the Chinese as a herbal remedy for fever and haemorrhoids [27,74,76]. It is structurally classified as an endoperoxide sesquiterpene lactone [28], with a

molecular weight of 282 and the chemical formula, $C_{15}H_{22}O_5$ [77,78], Fig (3). This sesquiterpene is stored in the trichomes on the *A. annua* leaf surface [76].

There are many semi-synthetic and synthetic artemisinin derivatives with the same biological activities as artemisinin. Some of these include artemether, arteether, and artesunate [76], Fig. (3). One problem with natural artemisinin is that it has low solubility in water and oil, limiting its therapeutic value. The advantage to using semi-synthetic analogues of artemisinin is that they have been engineered with pharmacological properties superior to those of the parent compound. These pharmacological properties include: improved solubility, potency, and more favourable metabolic and hydrolytic stabilities [76,79].

Biological Activities of Artemisinin

The disease for which artemisinin is most well known is malaria, a parasitic infection caused and transmitted by the mosquito *Plasmodium falciparum*. Malaria is considered the worst parasitic disease in developing parts of the world such as Africa and southeast Asia [27,28]. Traditionally, quinolines such as quinine, mefloquine, and amodiaquine have been used to treat malaria. However, over the years there has been an alarming rise in the number of quinoline-resistant parasites. Therefore, artemisinin has become the alternative option, although in the majority of cases it is used in combination with other anti-malarial drugs, such as these quinolines to avoid the emergence of resistant parasites before complete eradication of all the *P. falciparum* infection could be achieved [27]. Two great qualities of artemisinin are the low number of side effects [27] and a very rapid onset of action, in most cases clearing the patient's blood of parasites within 48h [74]. This latter quality is of particular interest in severe cases such as cerebral malaria [75]. In addition to its excellent anti-malarial activity, many artemisinin derivatives have been found to exhibit beneficial effects against other diseases such as hepatitis B, HIV, schistosomiasis, and certain cancers including breast, prostate, colon, and lung cancers, as well as leukemia [28]. Two specific examples of these diseases which can be treated by artemisinin are described below.



Artemisinin Artesunate

FIGURE 3 A comparison of parent compound, Artemisinin, to the structure of related trioxane derivative compound, Artesunate.

In an experiment by Jung and Shinzai, 1994, artemisinin-related trioxane derivatives, Fig. 3, were demonstrated as potential anti-HIV agents. The exact mechanism by which this anti-HIV activity occurs is still unknown. However, because artemisinin and its derivatives are producers of radical oxygen, it has been suggested that the activated oxygen species mediates the anti-HIV activity of the artemisinin-related compounds. The trioxane compound, which was found to have the greatest anti-viral activity, was 12-*n*-butyldeoxoartemisinin, but it only possessed this anti-HIV activity at a moderate degree [80].

There is evidence that monotherapy involving the artemisinin derivative, semi-synthetic artesunate, Fig. 3, has a very powerful effect against schistosomal infections. In an experiment by De Clercq *et al.,* artesunate was tested for its efficacy against *Schistosoma mansoni* infection. This study was carried out on a number of individuals infected with the trematode. Intensities of the infection's symptoms were reduced, as were the number of eggs found in patients' feces [81].

Although artemisinin has been used to treat several ailments, this sesquiterpene's greatest effect is against *P. falciparum*, and so it will likely continue to be used as part of combination therapy to treat malaria patients.

Biosynthesis of Artemisinin

Numerous studies over the last three decades have been dedicated to the elucidation of the artemisinin biosynthetic pathway. However, the complex pathway has not been conclusively outlined to date [76,82]. This section will describe the biosynthetic pathway of artemisinin, focussing on the branches containing two of the most probable direct precursors, artemisinic acid (ArtA) and dihydroartemisinic acid (DHA), Fig. 4.

Artemisinin biosynthesis occurs in the two outer apical cells of the secretory glandular trichomes of *A. annua* [83]. Starting from FPP, the biosynthetic pathway can be described in three phases. The first involves the conversion of FPP to amorpha-4,11-diene (AD), Fig. 4. In the second phase, AD is converted to DHA, and in the third and final phase, artemisinin is produced from DHA [76]. Beginning with phase one, the enzyme amorpha-4,11-diene synthase (ADS) cyclizes FPP to the bicyclic sesquiterpene, AD [76]. There are controversies over which of ArtA or DHA is actually a direct precursor to artemisinin [84]. Older literature has suggested that ArtA serves as the biogenic precursor [85,86]. Initially, it was believed that a P450 monooxygenase (CYP71AV1 in *A. annua*) was responsible for the three consecutive oxidations which convert AD to first artemisinic alcohol (ArtAlc), then to artemisinic aldehyde (ArtAld), and finally to ArtA, Fig 4. There has, however, been new evidence suggesting that AD is actually converted to DHA, starting with the same two oxidations mentioned above, yielding first ArtAlc and then ArtAld [84].

From this point, a recently identified enzyme, Dbr2 which codes for a double bond reductase 2, carries out the reduction of ArtAld to DHA [82]. Bertea

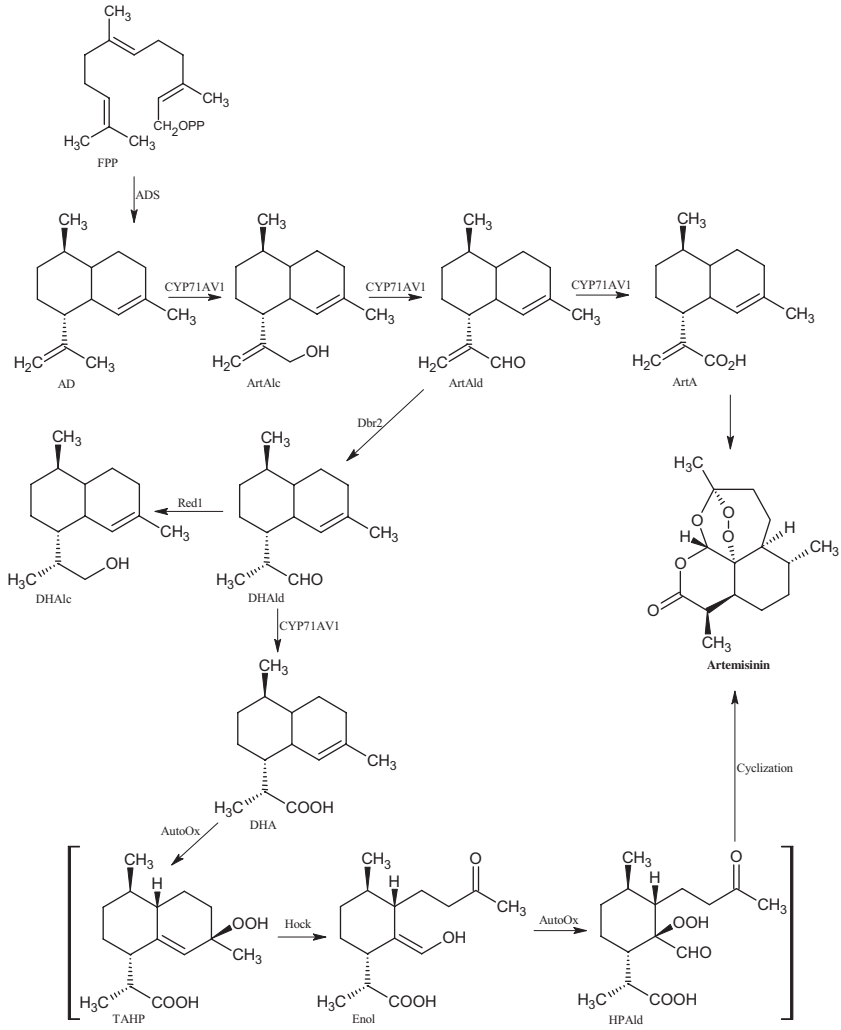


FIGURE 4 Two putative biosynthetic pathways of Artemisinin in *Artemisia annua*, including a proposed mechanism for the conversion of dihydroatemisinic acid to Artemisinin.

et al., 2005, have argued that there is no interconversion between ArtA and DHA, therefore ArtA could not be a good precursor candidate for artemisinin [84]. A recent experiment by Wallaart *et al.,* 2001, established that there is a correlation between the increase of artemisinin and the decrease of DHA during artemisinin biosynthesis, suggesting that DHA is indeed a direct precursor of the anti-malarial molecule [87]. Other research further supporting this, involved an experiment where a new artemisinin biosynthetic gene was identified. This gene (Red1) coded for a dihydroartemisinic aldehyde (DHAld) reductase. This

enzyme was found to have a negative impact on artemisinin production as it selectively converts DHAld to dihydroartemisinic alcohol (DHAlc). There was no detection of the reverse reaction taking place. Unlike the case of ADS which was only found to be actively transcribed in older leaves, Red1 transcription was high in both young and old leaves.

The biosynthetic steps of the final phase are still very controversial. The most recent views tend to favour the putative pathway from DHA to artemisinin through an allylic tertiary hydroperoxide intermediate (TAHP) [76], Fig. 4. It has been suggested that DHA acts as a singlet oxygen scavenger, protecting the plant cells from the damage of reactive oxygen species. During its quenching activity with singlet oxygen, DHA forms a hydroperoxide intermediate which has been proposed to be the factor enabling DHA's spontaneous conversion to artemisinin [87]. It was shown by Sy *et al.,* 2002, that DHA undergoes slow, non-enzymatic (spontaneous) autoxidation, even in the absence of light [88]. A mechanism for the spontaneous conversion of DHA to artemisinin was proposed by Sy and Brown in 2002. The formation of artemisinin's 1,2,4-trioxane ring begins with the oxygenation of DHA's double bond and this was found to be carried out by the same P450 enzyme, CYP71AV1, which oxidized AD to ArtA [89,90]. This yields the TAHP which then undergoes Hock cleavage, resulting in an enolic intermediate. This enol reacts with molecular oxygen to produce a vicinal hydroperoxyl-aldehyde (HPAld), Fig. 4, which is then cyclised, finally yielding artemisinin [90]. This mechanism was based on the production of peroxofabiane, a very similar compound analogous to artemisinin, also of interest in anti-malarial use, and found in *Fabiana imbricate* [91]. The majority of enzymes catalyzing the steps in these three phases have been cloned and characterized. These include ADS, CYP71AV1 (monooxygenase), Dbr2, and Red1, Fig. 4 [76,82,87,89,92,93].

Use of Biotechnology for Improved Production of Artemisinin

Due to the large number of individuals infected with *P. falciparum* in the world, artemisinin is in high demand. Unfortunately, it has low bioavailability (0.01–0.8% dry weight) in *A. annua,* making it very expensive and difficult to obtain [74]. Thus, there have been many efforts to find methods which can be used to successfully increase the production of artemisinin and its derivatives in *A. annua*, and thereby decrease its cost. Some of the methods developed include: the manipulation of genes in the MVA pathway, environmental conditions and elicitors, partial synthesis *via* microorganisms, and total synthesis.

Because the majority of the IPP used in *A. annua* for artemisinin production is a product of the MVA pathway, expression of the enzymes of this pathway have been manipulated in order to increase artemisinin yield. HMG-CoA reductase (HMGR) is a key enzyme of the MVA pathway, which regulates the carbon flux through the artemisinin route, thereby limiting its production. A very recent experiment by Nafis *et al.,* 2011, involved the overexpression

of this rate-limiting enzyme in *A. annua*, resulting in a significant increase in the overall artemisinin content of the transgenic *A. annua* plants [94]. The enzyme, FPPS, has also been successfully overexpressed in transgenic *A. annua* plants. Although FPPS is not a rate-limiting step of artemisinin synthesis, its overexpression improved artemisinin production up to 2.5 times in transformed plants [95].

In *A. annua*, FPP serves as a common precursor to two distinct and competing pathways, including steroid production and artemisinin synthesis. From FPP, steroid synthesis proceeds by the enzyme squalene synthase (SS), while artemisinin proceeds by ADS. When either of these enzymes is active, a sort of shunt is created, minimizing the carbon flux through the competing pathway [96]. From this, it was postulated that if the SS enzyme were to be downregulated, carbon flux would be focused on the artemisinin pathway, ultimately increasing its yield [97]. In an experiment by Yang *et al.,* 2008, transgenic *A. annua* plants expressing an antisense SS were developed. Although steroid production was impaired, artemisinin yield was not enhanced in these plants [96]. From this, Yang *et al.* suggested that the downregulation of SS narrowed the carbon flux through the squalene pathway, so that it could be redirected to other (unidentified) metabolic paths, but not necessarily artemisinin.

In a different experiment by Yang *et al,* 2010, it was shown that various environmental stress conditions can also have an effect on the regulation of artemisinin biosynthetic genes in *A. annua*. In many of the cases the stressors triggered upregulation of the biosynthetic genes. For example, exposure of the plant to UV light, which resulted in upregulation of ADS, ultimately resulted in greater artemisinin yield. Similarly, cold temperatures, which upregulated DXS expression, and hypoxia, which was seen to upregulate DBR2, were also found to improve artemisinin yields found in the senescent leaves of *A. annua*, as opposed to the younger leaves. This was due to the fact that the activities and levels of the enzymes affected by these environmental conditions, ADS and CYP71AV1 were higher in senescent leaves [98].

As an example of elicitors, one study used a phytohormone, salicylic acid (SA), which normally plays roles in plant growth and development, photosynthesis, defence, and transport within the vascular system. It was found to stimulate increased production of artemisinin in *A. annua*. The authors suggested that this increased artemisinin production occurred due to the burst of reactive oxygen species in response to SA, which in turn induced the conversion of DHA into artemisinin, as well as triggering an overall upregulation of many or all the genes of the artemisinin biosynthetic pathway [99]. In another example of elicitors, researchers used gibberellic acid (GA), a plant hormone which promotes plant germination and growth. It was in the study by Banyaii *et al.,* 2011, that GA was seen to trigger upregulation of three key enzymes in the artemisinin biosynthetic pathway: FPS, ADS and CYP71AV1 (monooxygenase). This ultimately resulted in a greater production of artemisinin by *A. annua* [100].

One increasingly popular biotechnological approach to increase artemisinin availability while simultaneously decreasing its cost is the use of genetically modified microorganisms, coupled with partial chemical synthesis. Together these methods provide scalability and cost efficiency, especially since certain microbes, *Saccharomyces cerevisiae*, for example, produce much greater amounts of advanced stage artemisinin precursors than the source plant, *A. annua* [101]. Over the last decade, several experiments found success in genetically modifying *S. cerevisiae* and *Escheria coli* to carry out partial synthesis of artemisinin [102]. For example, Anthony *et al.,* 2009, optimized the biosynthetic pathway of mevalonate-based isoprenoids. By increasing the promoter strength of mevalonate kinase and ADS, the researchers managed to produce AD in greater amounts, fivefold more, than what was originally possible for *E. coli* [103]. Once AD is produced it can be chemically converted to artemisinin in a relatively simple partial synthesis. In the case of *S. cerevisiae*, researchers have engineered a partial artemisinin biosynthetic pathway including AD and a new cytochrome P450 monooxygenase (CYP71AV1) which carries out a three step oxidation to yield ArtA. Furthermore, yeast export the ArtA product into their surrounding media, allowing for an inexpensive and easy purification system to retrieve the product of interest. As is the case with *E. coli*, from this point, partial synthesis could be employed to produce artemisinin [101].

The final method to be discussed is total synthesis of artemisinin, which usually begins with a stable and highly available monoterpene such as (R)-(+)-citronellal, Fig. 5, as was done by Xu *et al.,* 1986; or more recently, the monoterpene, (+)-isolimonene, Fig. 5, which was used by Yadav *et al.,* 2003 [104,105]. The monoterpenes are subjected to a number of chemical alterations which were determined by retrosynthetic analysis of artemisinin. Though this technique has been successful, it has thus far proved to be the least commercially feasible since it is expensive and lengthy; sometimes the number of steps required to achieve a fully functional artemisinin compound are upwards of 10 [104].

In summary, there is a great potential for the mass production of artemisinin through plant and microbial biotechnology. However, a commercially viable system for the large-scale production of this compound has not yet been developed. To achieve this goal, all genes involved in artemisinin biosynthesis must be first cloned and characterized.

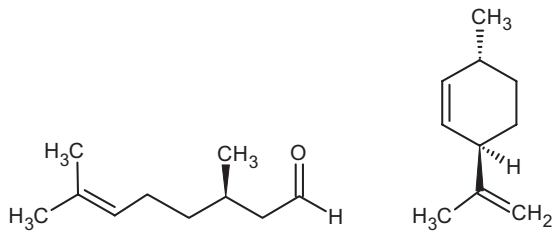


FIGURE 5 Chemical structures of (R)-(+)-citronellal, left, and (+)-isolimonene, right; stable monoterpenes which are used as starting points for total synthesis of artemisinin.

TAXOL (DITERPENE)

Taxol, commercially called Paclitaxel, is a natural diterpenoid product [106] which is found in the needles and bark of various members of the *Taxaceae* family. Among these members are *Taxus brevifolia*, or Pacific Yew [36] and *Taxus cuspidata*, or Japanese Yew [37]. Taxol was structurally defined by Wani *et al.* in 1971. Its chemical formula is $C_{47}H_{51}NO_{14}$ and it has a molar mass of 853 [107], Fig. 6. Taxol is one of the most structurally complex of the approximately 400 taxoid derivatives which have been identified to date [108]. This diterpene is composed of a terpenoid-derived core skeleton, which is highly modified by several hydroxylations and acylation reactions. Furthermore, two hydrolyzed esters are added onto allylic carbons 10 and 13 of the core molecule [107].

Biological Activities of Taxol

Taxus cuspidata, commonly known as the Japanese Yew, has been long used in China as an anti-diabetic, diuretic, and as an emmenagogue [37], but it is most well known for its use as a highly effective chemotherapeutic agent against advanced stage localized metastatic breast cancer [36]. Its mode of action involves its binding to the polymerized tubulin, and triggering microtubule assembly, thus inhibiting their depolymerisation, which would be needed for mitosis to occur [106]. In some cases, however, cancer cells being treated with paclitaxel can develop a resistance to the drug, and for that reason it is usually given in combination with other drugs (e.g., biricodar, Incel) that restore paclitaxel sensitivity [106]. Taxol is also effective against several other cancers such as ovarian cancer and leukemia [107,108].

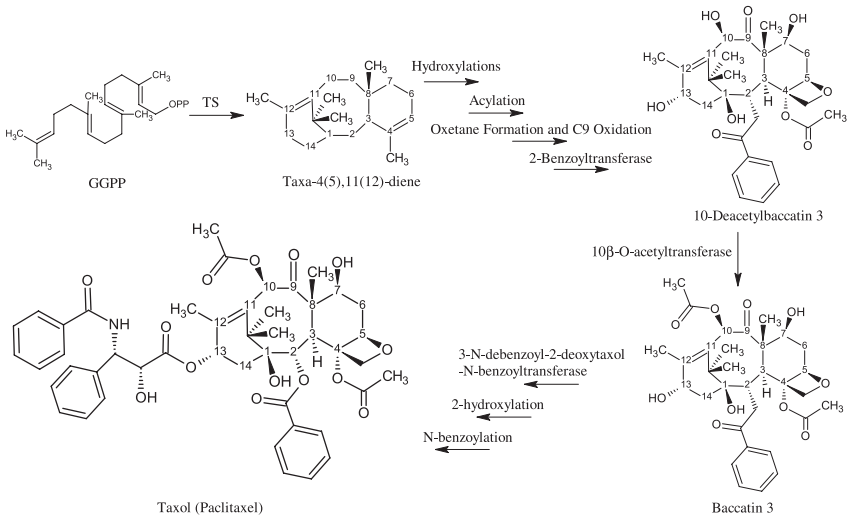


FIGURE 6 Putative biosynthetic pathway of taxol (paclitaxel) in *Taxus* species.

The precise role of taxol in *Taxus* species is not clear. From taxol's anastomosing network of biosynthetic pathways, having several routes by which taxol can be achieved, it can be postulated that perhaps it is not directly produced by the tree for any specific duty. Instead it may be that taxol is synthesized as a by-product, during the plant's production of defence chemicals. This postulate was made based on observations that a plant's defence response is upregulated in damaged cells. For example, in one study it was found that Taxol production was upregulated in *Taxus chinensis* cell cultures, where the cells were damaged by ultrasound treatment [109].

Biosynthesis of Taxol

As with all diterpenes, the IPP precursor for taxol biosynthesis is provided by the plastidial MEP pathway [37]. The parent prenyl diphosphate which goes on to produce taxol is GGPP, which is biosynthesized by the condensation of one DMAPP and three IPP isoprene units (as was described earlier). From this point there are various anastomosing pathways which can be followed, resulting in slightly different taxoid chemical species. In fact, there are approximately 400 different taxoids which are produced by the *Taxus* species, though the taxoid species of medical interest is taxol (paclitaxel), and thus its biosynthesis will be the focus of this section [110].

Beginning with GGPP, an enzyme called taxadiene synthase (TS) catalyzes the cyclization of the prenyl diphosphate to taxa-4(5),11(12)-diene (taxadiene), Fig. 6 [37,111]. This marks the committed step of taxol biosynthesis [110]. Following this, are several hydroxylations, carried out by cytochrome P450-type enzymes [111], which occur at various carbon positions, for example, C_1, C_2, C_5, C_7, C_9, C_{10}, and C_{13} [112]. Following the hydroxylations there is a coenzyme A dependent acylation which yields a taxoid intermediate with an acetyl group on C_5 [110,112]. This is an important step as this acetyl group will go on to become part of taxol's characteristic oxetane ring, which is still being investigated for its potential role in taxol's anti-cancer activity [113]. Along with oxetane formation, is C_9 oxidation followed by the addition of a benzoyl group at C_2, catalyzed by 2-benzoyltransferase. This yields the highly abundant taxol precursor, 10-deacetylbaccatin 3, Fig. 6 [110].

From this point, an enzyme called 10β-O-acetyltransferase carries out the conversion of a hydroxyl group at C_{10} to an acetyl group, resulting in the production of another highly abundant late stage precursor, baccatin 3, Fig. 6 [110]. Due to the large number of routes which a taxoid can follow there are many different, but closely similar, 10-deacetylbaccatin three derivatives, approximately 33, which serve as excellent late stage precursor compounds to be extracted for production of this valuable drug [114] (to be discussed later). In the final steps of taxol biosynthesis a 3-N-debenzoyl group is added onto the hydroxyl at C_{13} by way of an enzyme, 3-N-debenzoyl-2-deoxytaxol-N-benzoyltransferase. The amino group on the C_{13} side chain is then benzoylated (termed N-benzoylation

in Fig. 6), and finally the second carbon on the side chain is hydroxylated, yielding the highly desired product, taxol [110,115].

Use of Biotechnology for Improved Production of Taxol

The diterpene, Taxol was traditionally obtained by extraction from the needles and bark of the *Taxus* species (Yew trees) [116]. The amount of taxol that can be extracted from these source trees is very low, 1g per 30lbs of bark, while treatment of a cancer patient requires an average of 2g of taxol to be administered [117]. In order to obtain enough taxol for treatment of one cancer patient, approximately 12 large *T. brevifolia* trees must be cut down [117]. This problem of limited natural taxol source gave rise to what has been termed "the Taxol Dilemma". Recently, within the last couple of decades, there have been several routes taken in an effort to overcome this Taxol Dilemma. Those techniques include: cell culture, semi-synthesis, and biosynthesis in microorganisms.

In one example of biosynthesis in microorganisms, researchers have turned to taking advantage of a yew tree fungus, *Taxomyces andreanae*, a fungal endophyte which grows in the bark of *T. brevifolia* and was discovered to produce taxol, although not in very significant amounts [118]. Later discovered was another fungal microbe, *Pestalotiopsis microspora*, which resides in the bark of the Himalayan Yew (*Taxus wallachiana*). It was found to produce a taxol compound which exhibited anti-cancer activity and accumulated in culture at a "micrograms per liter" level which was sufficiently significant for this fungus to be of use in fermentation technology [118]. Another microorganism which has been used to solve the Taxol Dilemma is *S. cerevisiae*, in an experiment which involved the combined overexpression of two taxol biosynthetic enzymes, including geranyl geranyl diphosphate synthase (GGPPS) and TS. Their results showed a significant increase, 50%, in taxadiene production by the yeast cells. From this late stage precursor the rest of the taxol synthetic pathway could be chemically completed [78], leading to the next method used to overcome the Taxol Dilemma, total synthesis.

Because total synthesis of taxol is such a costly and complicated procedure, researchers have turned to semi-synthesis, beginning with extraction of advanced stage precursor compounds and then performing relatively minor chemical alterations on them to produce taxol [119]. One such example is that of the four step semi-synthesis, which begins with protection of the hydroxyl group at C_7 of 10-deacetylbaccatin III (10-DABIII) by addition of the regioselectively protecting agent, 1,1'-thiocarbonyldiimidazole [119]. This protection step was followed by acetylation of the protected 10-DABIII to yield 7-(imidazolyl)-thiocarbonyl-baccatin III. Thirdly there was an esterification of the (2R,3S)-phenylisoserine side chain with 7-(imidazolyl)-thiocarbonyl-baccatin III to produce the protected taxol, which upon removal of the protective agent *via* addition of *p*-toluenesulfonic acid and aqueous hydrochloride in methanol, yielded the final product of interest, taxol [118]. Using semi-synthesis such as

that recently described, eliminates the need to extract taxol from large numbers of Yew trees, and also the need to synthesize taxol completely *de novo*. Instead, advanced stage precursors, which are more readily available in the needles of various *Taxus* species, can be extracted and used for semi-synthesis. Extraction of late stage precursors is not only limited to Yew trees but they can also be obtained from *Taxus* cell cultures.

Plant cell cultures have been optimized for easy regeneration and induction of Taxol biosynthesis. There are several different elicitors which can be added to the cultures for enhanced taxol production and some of these include: methyl jasmonate, Cerium (Ce^{4+}), chitosan, fungal carbohydrates, SA, and yeast extract [109,120,121]. One experiment found that the combined use of a mechanical stimulus, ultrasound, and methyl jasmonate elicitor resulted in 20–50% higher yields of taxol in *T. chinensis* cell suspension culture than when either of these treatments were used alone [109]. Another group of researchers saw a 16-fold increase in the production of taxol in a *Taxus baccata* suspension cell culture upon addition of biomass growth factors and elicitor mixtures including: methyl jasmonate, SA, and a fungal elicitor [121].

Together these biotechnological mechanisms have helped to overcome the problem of limited natural taxol source. However, as the demand for taxol continues to grow, the search for more efficient and more inexpensive taxol production systems must also continue.

GINSENOSIDES (TRITERPENES)

Ginsenosides are members of a class of triterpenes known as saponins, which play primarily defence roles as anti-microbials, insecticidals, anti-fungals, and virucidals in plants [122]. Avencins are a good example of saponins which act as anti-microbials against *Gaeumannomyces graminis*, a soil dwelling pathogen that attacks the roots of oat plants [123].

Ginsenosides are the major bioactive molecules of the various Ginseng plants which belong to the *Araliaceae* family. Within these plants the ginsenosides are found to be localized to the roots [41,124,125]. Within this family are included *Panax ginseng*, Korean Ginseng [40], and *Panax quinquefolius*, American Ginseng, both of which are perennial herbs from the *Araliaceae* family [124]. Structurally, ginsenosides are tetracyclic triterpene (dammarane) glycosides with a C_{20} modified side chain. They are labelled ginsenoside Rx according to their polarity, which decreases from "Ra" to "Rh" [41]. Ginsenosides differ in the type and number of sugar moieties and the position at which these moieties are attached [41]. They are organized into various sub-classes according to their aglycone structures [124]. Two of the main groups include the dammarane and oleanane type ginsenosides [124], Fig. 7.

Ginseng is commercially available as white and red roots. Those roots which are steamed, dried, and unpeeled are referred to as the red roots, whereas those which are peeled and dried without steaming are called the white roots [41].

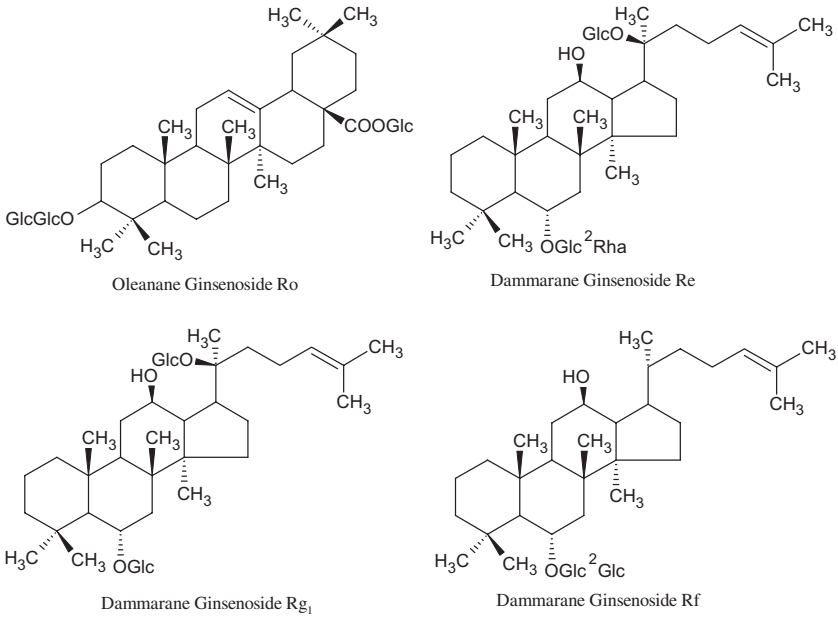


FIGURE 7 One oleanane type and three dammarane type triterpene saponins (ginsenosides) found most abundantly in *P. ginseng*.

Biological Activities of Ginsenosides

In general, ginseng extracts are considered adaptogenic agents that improve vitality, enhance resistance to stress and ageing, and increase physical performance [40]. Ginsenosides also have several specific medicinal uses including anti-diabetic, anti-hypertension, anti-cancer, anti-arthritic, and anti-platelet activities [41,42,43,126]. One experiment tested the effects of the total ginsenosides from *P. ginseng* on hyperglycemic induced mice. It was found that after oral administration of these total ginsenosides the blood glucose levels were dramatically decreased in the mice. These results showed promising evidence that ginsenosides could be of use in the treatment of diabetes patients [42]. Ginsenosides, such as ginsenoside Rh2 from *P. ginseng*, also have been shown to have anti-cancer properties, as they can inhibit cell cycle progression in cancer cells [126]. Certain ginsenosides have also been found effective against cancers which have developed multi-drug resistance. Multi-drug resistance is caused by an ABC transporter (MDR1) which evicts therapeutic agents from cells, depriving them of treatment. The use of ginsenosides (specifically ginsenoside Rd from *P. ginseng*) increased the ubiquination of MDR1, leading to its degradation. This ultimately resulted in the retention of anti-cancer agents such as taxol within the breast cancer cells [126]. Therefore, it was concluded that ginsenoside Rd is effective for

use together with anti-cancer agents to downregulate multi-drug resistance in breast cancer cells [126].

Biosynthesis of Ginsenosides

Starting from FPP, the biosynthesis of ginsenosides begins with the condensation of two FPP molecules into squalene – a reaction catalyzed by SS, Fig. 8 – which is subsequently oxidized to 2,3-oxidosqualene by squalene epoxidase (SQE) [127]. This serves as the biosynthetic branch point for ginsenosides and phytosterols. The following cyclization reactions of 2,3-oxidosqualene ultimately yield the triterpene saponins [124,128]. Oxidosqualene is then cyclized to a variety of different cyclic derivatives. The type of cyclic skeleton that is formed depends on the type of cyclase that carries out the reaction [128]. Of the many cyclases which have been identified and characterized, the three main enzymes include dammarenediol synthase (DS) which forms dammarane type saponins, β-amyrin synthase (βAS), which gives rise to oleanane type saponins, and α-amyrin synthase (αAS), which produces ursane type saponins [129], Fig. 8.

What lies downstream of the cyclization step remains largely unknown [124]. Although there is no concrete evidence as of yet, it has been proposed that

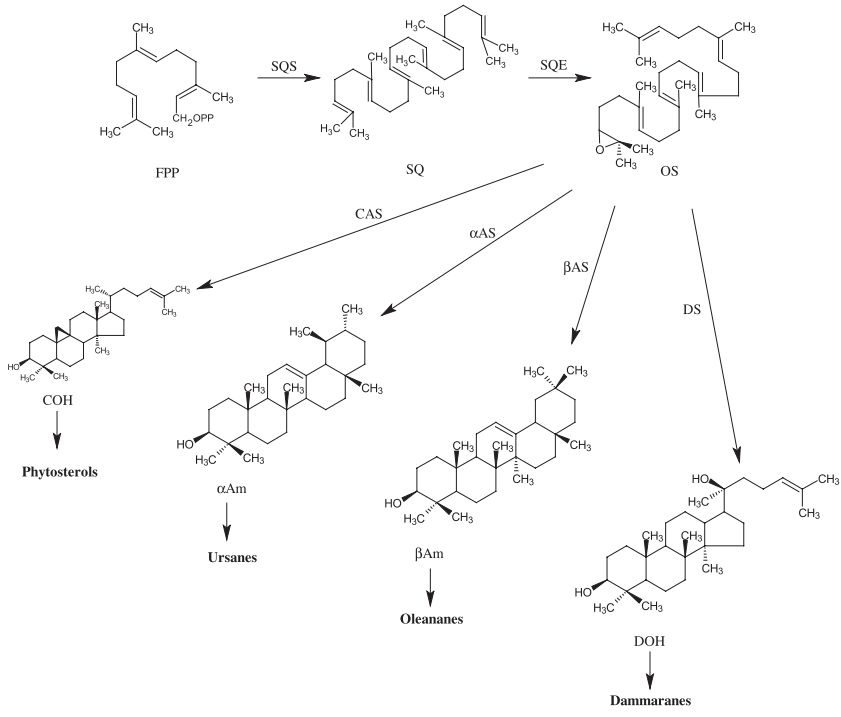


FIGURE 8 Putative biosynthetic pathway of ginsenosides in *Panax ginseng*.

certain CYP450 enzymes, UDP-glycosyltransferases, and other enzymes play key roles in many oxidations, hydroxylations, and glycosylations which result in the conversion of β-amyrin and dammarenediol into various ginsenosides [127,128]. Generally, during the biosynthesis of secondary metabolites the glycosylation step is last [124]. The sugar side chain addition gives the resulting molecule greater stability and increased water solubility [130]; it is also usually responsible for giving many saponins their biological activity [128].

Use of Biotechnology for Improved Production of Ginsenosides

Due to the many medicinal uses of ginsenosides, the desire to elucidate their biosynthetic pathway and improve their yield by metabolic engineering has recently intensified. Farnesyl diphosphate synthase (FPPS) was recently identified as a key regulatory enzyme in both phytosterol and triterpene biosynthesis. In an experiment by Kim *et al.* in 2010, FPPS was overexpressed in the hairy roots of *Centella asiatica*. The upregulation of FPPS did increase the flux of carbon through that early step (GPP to FPP) and resulted in a greater triterpene saponin yield indicating that triterpene yield may indeed be enhanced through biotechnology [131].

In another experiment, the plant growth regulator, methyl jasmonate, was used to induce saponin production in plants overexpressing the SS gene [125]. Hormone treatment resulted in a subsequent increase in expression of two enzymes downstream of SS, including SQE and β-amyrin synthase (βAM). The results proved that enhanced SS activity could successfully yield greater ginsenoside and phytosterol products in *P. ginseng* roots treated with methyl jasmonates, confirming a vital role for SS as a regulatory enzyme for triterpene biosynthesis [125].

As illustrated in Fig. 8, the 2,3-oxidosqualene metabolite is a branch point between the phytosterol and saponin pathways. Cycloartenol synthase (CAS) is the enzyme responsible for conversion of OS to cycloartenol, the first specific precursor in the production of phytosterols. In the other branch, DS is the enzyme which directs OS down the saponin pathway [125]. As reported by Liang *et al.,* 2009, the ginsenoside yield in *P. ginseng* hairy roots was enhanced by antisense suppression of the CAS gene. This CAS suppression resulted in upregulation of DS, which ultimately led to the greater ginsenoside production in *P. ginseng* [132]. Due to its medicinal applications and high cost, efforts to improve ginsenosides production in plants continue.

CAROTENOIDS (TETRATERPENES)

In nature there are approximately 700 members of the carotenoid family and of those about 20–50 are part of the human diet [133,134]. Carotenoids belong to the tetraterpenoid group of natural products with their general structure shown in Fig. 9 [133]. They have a conjugated double bond hydrocarbon

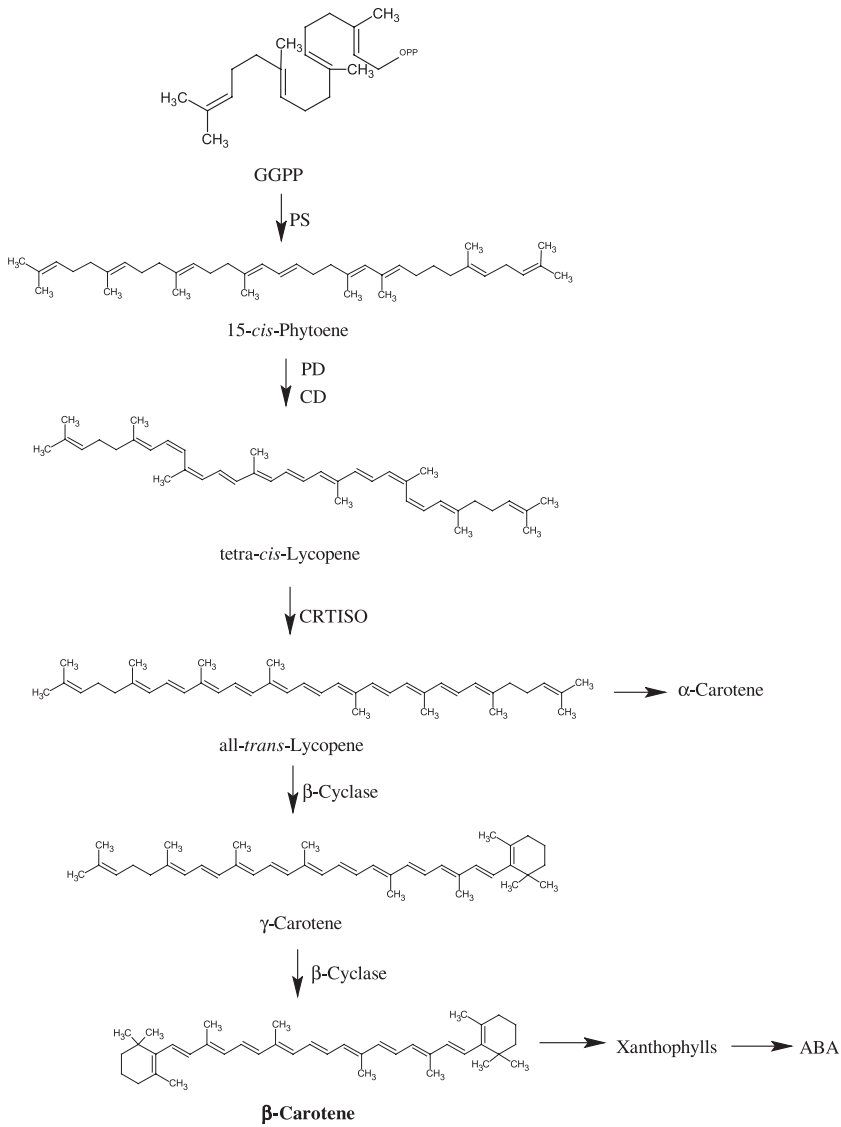


FIGURE 9 Biosynthetic pathway of the tetraterpene carotenoid, β-carotene in higher plants.

backbone [3] and can have either β- or ε-rings added to one or both ends of this polyene chain [133]. These include α- and β-carotene; the latter which has one β-ring on both the ends and the former which has one of each ring on either end. These carotenes can be further hydroxylated, yielding xanthophylls, which eventually give rise to the important plant growth regulators, such as ABA.

Carotenoids are synthesized and accumulated in all plastids of higher plants [133]. They are also found as pigmentation molecules in various birds, crustaceans, and fish, though they are not synthesized in those organisms but rather obtained from plant materials *via* diet [135]. Carotenes range in colours from pale yellow, ζ-carotene, to deep red, lycopene, and this colouration depends on the number of conjugated double bonds within the hydrocarbon backbone of the molecule. As the number of conjugated double bonds increases, the colour of the carotene darkens [136].

Beta-carotene belongs to a structural group of carotenoids which have unsubstituted β-ring end groups, giving these carotenes their provitamin A activity. Other carotenes within this provitamin A group include α-carotene and β-cryptoxanthin [134]. Due to the presence of two unsubstituted β-rings, β-carotene has two times the provitamin A activity as the other two carotenoids [134]. It is found in great abundance (48–84% of total carotenoids) in fruit-bearing plants like Mango, *Mangifera indica*, and roots such as Carrot *Daucus carota*, giving them their orange-yellow colouration [137]. Beta-carotene is also found in certain orange root tubers such as sweet potato, *Ipomoea batatas* [138], and in the amyloplasts of starchy seeds such as those of Maize, *Zea mays* [133].

Biological Activities of Carotenoids (e.g., Beta-carotene)

The carotenoid compounds serve a wide array of biological functions in plants. One of the primary functions is as accessory pigments in the photosynthetic system [133]. They also protect membranes in the plant from photo-oxidative damage due to excess light energy by quenching triplet chlorophyll and singlet oxygen, and scavenging any other reactive oxygen species [139]. This quenching and scavenging ability of carotenoids is due to their conjugated double bonds [140]. Carotenes are also precursors to certain odour molecules and hormones such as ABA [133], attractants for vectors of pollination and seed dispersal, and antioxidants [11].

In humans, carotenoids can prevent certain eye and cardiovascular diseases. Age-related macular degeneration is a condition which affects elderly people, progressively impairing their vision until blindness. It has been suggested that oxidative stress is the factor leading to development of this disease, and therefore carotenoids would be useful against progression of macular degeneration due to their antioxidant properties [47]. One experiment tested the antioxidant effects of a cocktail including various carotenoid compounds (among these was β-carotene), on elderly persons with early and late stages of macular degeneration. The results showed that increased (added *via* diet) serum concentrations of the carotenoid mixture led to a decrease in the prevalence of late age-related macular degeneration [47]. Beta-carotene can also provide protection against UV irradiation, when used in conjunction with other carotenoids such as tocopherol, Table 1, and are used in orally administered sun protectants [11].

Another group of carotene-related compounds – the fat soluble molecules, tocopherols (Vitamin E) – also exhibit potent antioxidant activities [45]. There are different forms of tocopherols, with the most biologically active being α-tocopherol. Vitamin E is able to lower blood pressure levels in people with mild hypertension after regular use over a prolonged period. The mechanism by which this occurs has been suggested to be the neutralization of superoxides by the antioxidants [46].

Another group of carotenes have vitamin A activity, and are essential for human health. Vitamin A deficiency is a very large problem in certain developing areas of the world due to poor diet. In cases of severe vitamin A deficiency, inflicted persons can develop xerophthalmia which usually results in blindness, or they can die. Beta-carotene is enzymatically cleaved in the intestinal lumen to produce two molecules of vitamin A. With this knowledge, β-carotene is used as a supplement to treat vitamin A deficiency [134,141].

Biosynthesis of Carotenoids (e.g., Beta-carotene)

Carotenoid biosynthesis is carried out in all plastid types, and so they are found in all tissues of all higher plants, albeit at greatly varied concentrations [133]. In the plastids, a short chain prenyl transferase, GGPPS, catalyzes the condensation of three IPP molecules with DMAPP to produce the first parent precursor giving rise to both the di- and tetraterpene molecules, GGPP [9]. The key regulatory step of carotene biosynthesis involves the condensation of two GGPP molecules by phytoene synthase (PS) [135] to produce the colourless 40-carbon compound, 15-*cis*-phytoene, Fig. 9. PS is responsible for controlling the flow of carbon through the carotenoid biosynthetic pathway [136]. Four desaturation reactions, introducing two symmetric double bonds, are mediated by phytoene desaturase (PD) and ζ-carotene desaturase (CD) which converts phytoene to the red pigment, tetra-*cis*-lycopene. Cyclases prefer an all-*trans* substrate, therefore a carotenoid isomerase, CRTISO, catalyzes the isomerisation of tetra-*cis*-lycopene to all-*trans*-lycopene [142]. The following cyclization step is a branch point where lycopene [133] gives rise to the orange and yellow pigments by being either cyclized to α-carotene or β-carotene [134]. There are two cyclase enzymes which carry out either branch, ε- and/or β-cyclase [134]. In the pathway to β-carotene, only the β-cyclase is involved, mediating the addition of a β-ring onto either end of the lycopene molecule. In the pathway to α-carotene, it is both the β- and ε-cyclases which catalyze the addition of a ε-ring at one end, and a β-ring at the other end of lycopene [133].

Use of Biotechnology for Improved Production of Carotenoids: The Case of Provitamin A

Due to the recent growth of the vitamin A deficiency problem in developing countries, there is ample interest in increasing the production of provitamin

A (β-carotene), in a cost efficient manner. Described below, are some genetic engineering and agricultural techniques which have been employed to achieve this. One example of genetic manipulation involved the overexpression of PS in canola, *Brassica napus*, leading to 50 times greater carotenoid production in the oilseeds of this plant [136].

In another experiment the entire biosynthetic pathway of β-carotene was introduced into rice, *Oryza sativa*. This was done by coupling the overexpression of PS from daffodil, *Narcissus pseudonarcissus*, with that of the bacterial crtI gene, and the lycopene β-cyclase gene. The crtI gene is responsible for mediating the four desaturation reactions during the conversion of the colourless phytoene to red lycopene in bacteria. It was found that together, overexpression of these enzymes yielded greater production of β-carotene in this strain of rice, which came to be known as Golden Rice [143]. There was later a strain developed called Golden Rice 2, which was an improved version of the original Golden Rice. This improvement was achieved by introducing a PS gene from Maize, rather than from daffodil, as was the case with the original Golden Rice. These genetic manipulations resulted in a 23-fold increase in production of total carotenes, specifically β-carotene, than that found in the original Golden Rice plants [144].

Not only can production of carotenoids be improved in plants by way of genetic engineering; other simpler methods have been employed. In the case of an experiment by Rivas *et al.,* 2011, the carotenoid content, including β-carotene, in the leaves of Citrus trees (*Citrus*) were increased in response to girdling. Girdling is an agricultural technique commonly practised to increase the accumulation of sugars and carotenoids in fruit. Because girdling causes oxidative stress in the plant, certain protective antioxidant compounds, in this case, carotenoids, are upregulated to protect the tree from oxidative damage [140].

Though practises such as girdling are simpler and cheaper techniques by which to increase carotenoid production in plants, biotechnological methods such as upregulation of certain key enzymes have yielded far better results, and thus, efforts shall continue in the search to develop the most efficient genetic manipulations, producing the greatest carotenoid content at the lowest cost possible.

ACKNOWLEDGEMENT

The authors are grateful to Zerihun Demissie for his contribution during the validation of molecular structures produced for this manuscript.

ABBREVIATIONS

ABA	abscisic acid
ACoA	acetyl CoA
AACT	acetyl CoA-c-transferase
AD	amorpha-4,11-diene

ADS	amorpha-4,11-diene synthase
AutoOx	auto-oxidation
αAm	α-amyrin
αAS	α-amyrin synthase
βAm	β-amyrin
βAS	β-amyrin synthase
ArtA	artemisinic acid
ArtAlc	artemisinic alcohol
ArtAld	artemisinic aldehyde
CD	ζ-carotene desaturase
CRTISO	carotenoid isomerase
COH	cycloartenol
CAS	cycloartenol synthase
DOH	dammarenediol
DS	dammarenediol synthase
DHA	dihydroartemisinic acid
DHAlc	dihydroartemisinic alcohol
DHAld	dihydroartemisinic aldehyde
DMAPP	dimethyl allyl diphosphate
CDP-ME	4-diphosphocytidylyl-methylerythritol
CMK	CDP-ME kinase
CDP-MEP	4-diphosphocytidylyl-methylerythritol-2-phosphate
DXP	deoxyxylulose-5-phosphate
Dbr2	double bond reductase 2
DXR	DXP reductoisomerase
DXS	DXP synthase
FPP	farnesyl diphosphate
FPPS	farnesyl diphosphate synthase
GPP	geranyl diphosphate
GPPS	geranyl diphosphate synthase
GGPP	geranyl geranyl diphosphate
GGPPS	geranyl geranyl diphosphate synthase
Glc	glucose
G3P	glyceraldehyde-3-phosphate
HPAld	hydroperoxyl-aldehyde
HMBPP	hydroxymethylbutenyl-4-diphosphate
HDR	HMBPP reductase
HDS	HMBPP synthase
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA
HMGS	3-hydroxy-3-methylglutaryl-CoA synthase
HMGR	HMG-CoA reductase
Hock	hock cleavage
IPD	(−)-*trans*-isopiperitenol dehydrogenase
IPI	(+)-*cis*-isopulegone isomerase
IPP	isopentyl diphosphate
IPPI	isopentyl diphosphate isomerase
IPR	(−)-isopiperitenone reductase
L3H	limonene-3-hydroxylase

LS	limonene synthase
MFS	menthofuran synthase
MR	(−)-menthone reductase
ME-cPP	methylerythritol-2,4-cyclodiphosphate
MDS	ME-cPP synthase
MEP	methyerythritol-4-phosphate
MCT	MEP cytidylyltransferase
MVA	mevalonate
MDC	mevalonate-5-diphosphate decarboxylase
MK	mevalonate kinase
MDR1	multi-drug resistance 1
OS	2,3-Oxidosqualene
PMK	phosphomevalonate kinase
PD	phytoene desaturase
PS	phytoene synthase
PGP-HSF	plant growth promoter-hyaline sterile fungus
PDR	pleiotropic drug resistance
PR	pulegone reductase
α/β-Phe	α/β-phenylalanine
Rha	rhamnopyranose
SQ	squalene
SQS	squalene synthase
SQE	squalene epoxidase
TAHP	allylic tertiary hydroperoxide intermediate
TRPM8	transient receptor potential cation channel subfamily M member 8
TS	taxadiene synthase

REFERENCES

[1] D.J. Mcgarvey, R. Croteau, Plant Cell 7 (1995) 1015–1026.
[2] K. Yazaki, FEBS Lett. 580 (2006) 1183–1191.
[3] B. Buchanan, W. Gruissem, R. Jones, Biochemistry and Molecular Biology of Plants. Wiley, 2002.
[4] B. Adorjan, G. Buchbauer, Flavour Fragrance J. 25 (2010) 407–426.
[5] M. Rodriguez-Concepcion, Arch. Biochem. Biophys. 504 (2010) 118–122.
[6] T. Hasunuma, S. Takeno, S. Hayashi, M. Sendai, T. Bamba, S. Yoshimura, J. Biosci. Bioeng. 105 (2008) 518–526.
[7] D. Ganjewala, S. Kumar, R. Luthra, Curr. Issues Mol. Biol. 11 (2009) i35–i45.
[8] D.A. Nagegowda, FEBS Lett. 584 (2010) 2965–2973.
[9] S.S. Mahmoud, R.B. Croteau, Trends Plant Sci. 7 (2002) 366–373.
[10] F. Bakkali, S. Averbeck, D. Averbeck, M. Waomar, Food Chem. Toxicol. 46 (2008) 446–475.
[11] P.D. Fraser, P.M. Bramley, Prog. Lipid Res. 43 (2004) 228–265.
[12] A.K. Neelakandan, H.T.M. Nguyen, R. Kumar, L.P. Tran, S.K. Guttikonda, T.N. Quach, Plant Mol. Biol. 74 (2010) 503–518.
[13] A. Cecilia Mesa-Arango, J. Montiel-Ramos, B. Zapata, C. Duran, L. Betancur-Galvis, E. Stashenko, Mem. Inst. Oswaldo Cruz 104 (2009) 878–884.
[14] A.F. Barrero, L.F.Q. del Moral, A. Lara, M.M. Herrador, Planta Med. 71 (2005) 67–71.

[15] A. Ulubelen, Phytochemistry 64 (2003) 395–399.
[16] J. Bruin, M. Dicke, M.W. Sabelis, Experientia 48 (1992) 525–529.
[17] R. Mumm, M.A. Posthumus, M. Dicke, Plant Cell Environ. 31 (2008) 575–585.
[18] F.A. Santos, V.S.N. Rao, Phytother. Res. 14 (2000) 240–244.
[19] M.A. Sherkheli, H. Benecke, J.F. Doerner, O. Kletke, A.K. Vogt-Eisele, G. Gisselmann, J. Pharm. Pharm. Sci. 12 (2009) 116–128.
[20] M.H. Boskabady, Z. Jafari, I. Pouraboli, Phytother. Res. 25 (2011) 530–535.
[21] A.T. Peana, A.G. De Montis, S. Sechi, G. Sircana, P.S. D'Aquila, P. Pippia, Eur. J. Pharmacol. 497 (2004) 279–284.
[22] G. Woronuk, Z. Demissie, M. Rheault, S. Mahmoud, Planta. Med. 77 (2011) 7–15.
[23] R. Eccles, J. Pharm. Pharmacol. 46 (1994) 618–630.
[24] A. Calcabrini, A. Stringaro, L. Toccacieli, S. Meschini, M. Marra, M. Colone, J. Invest. Dermatol. 122 (2004) 349–360.
[25] P.C. Braga, M. Dal Sasso, M. Culici, T. Bianchi, L. Bordoni, L. Marabini, Pharmacology 77 (2006) 130–136.
[26] E.S. Fernandes, G.F. Passos, R. Medeiros, F.M.d. Cunha, J. Ferreira, M.M. Campos, Eur. J. Pharmacol. 569 (2007) 228–236.
[27] B. Witkowski, J. Lelievre, M.J.L. Barragan, V. Laurent, X. Su, A. Berry, Antimicrobial. Agents Chemother. 54 (2010) 1872–1877.
[28] C. Lei, D. Ma, G. Pu, X. Qiu, Z. Du, H. Wang, Ind. Crop. Prod. 33 (2011) 176–182.
[29] J. Gertsch, M. Leonti, S. Raduner, I. Racz, J. Chen, X. Xie, Proc. Natl. Acad. Sci. 105 (2008) 9099–9104.
[30] J.H. Joo, A.M. Jetten, Cancer Lett. 287 (2010) 123–135.
[31] B. Schilter, I. Perrin, C. Cavin, A.C. Huggett, Carcinogenesis 17 (1996) 2377–2384.
[32] V. Sridevi, P. Giridhar, G.A. Ravishankar, Curr. Sci. 99 (2010) 1101–1104.
[33] J.P. Barbosa, V.L. Teixeira, R. Villaca, R.C. Pereira, J.L. Abrantes, I.C.P.D. Frugulhetti, Biochem. Syst. Ecol. 31 (2003) 1451–1453.
[34] H.S. Bodiwala, S. Sabde, D. Mitra, K.K. Bhutani, I.P. Singh, Nat. Prod. Commun. 4 (2009) 1173–1175.
[35] M.P. Gupta, M. Gupta, A.F.R. Stewart, R. Zak, Biochem. Biophys. Res. Commun. 174 (1991) 1196–1203.
[36] F.A. Holmes, R.S. Walters, R.L. Theriault, A.D. Forman, L.K. Newton, M.N. Raber, J. Natl. Cancer Inst. 83 (1991) 1797–1805.
[37] Q. Wu, C. Sun, H. Luo, Y. Li, Y. Niu, Y. Sun, Planta Med. 77 (2011) 394–400.
[38] D. Yu, Y. Sakurai, C. Chen, F. Chang, L. Huang, Y. Kashiwada, J. Med. Chem. 49 (2006) 5462–5469.
[39] E. Selzer, E. Pimentel, W. Wacheck, W. Schlegel, H. Pehamberger, B. Jansen, J. Invest. Dermatol. 114 (2000) 935–940.
[40] M. O'Hara, D. Kiefer, K. Farrell, K. Kemper, Arch. Fam. Med. 7 (1998) 523–536.
[41] J.D. Park, D.K. Rhee, Y.H. Lee, Phytoch. Rev. 4 (2005) 159–175.
[42] J.T. Xie, C.Z. Wang, A.B. Wang, J. Wu, D. Basila, C.S. Yuan, Acta Pharmacol. Sin. 26 (2005) 1104–1110.
[43] K.R. Kim, T.Y. Chung, H. Shin, S.H. Son, K. Park, J. Choi, Biol. Pharm. Bull. 33 (2010) 604–610.
[44] T.J. Smith, Expert Opin. Investig. Drugs 9 (2000) 1841–1848.
[45] S. Werner, V. Boehm, J. Agric. Food Chem. 59 (2011) 1163–1170.
[46] M. Boshtam, M. Rafiei, K. Sadeghi, N. Sarraf-Zadegan, Int. J. Vitam. Nutr. Res. 72 (2002) 309–314.

[47] T. Michikawa, S. Ishida, Y. Nishiwaki, Y. Kikuchi, T. Tsuboi, K. Hosoda, Asia Pac. J. Clin. Nutr. 18 (2009) 1–7.
[48] S. Richer, W. Stiles, L. Statkute, J. Pulido, J. Frankowski, D. Rudy, Optics 75 (2004) 216–229.
[49] P. Di Mascio, S. Kaiser, H. Sies, Arch. Biochem. Biophys. 274 (1989) 532–538.
[50] E. Giovannucci, A. Ascherio, E.B. Rimm, M.J. Stampfer, G.A. Colditz, W.C. Willett, J. Natl. Cancer Inst. 87 (1995) 1767–1776.
[51] T. Patel, Y. Ishiuji, G. Yosipovitch, J. Am. Acad. Dermatol. 57 (2007) 873–878.
[52] E. Herro, S.E. Jacob, Dermatitis 21 (2010) 327–329.
[53] N. Galeotti, L.D. Mannelli, G. Mazzanti, A. Bartolini, C. Ghelardini, Neurosci. Lett. 322 (2002) 145–148.
[54] A. Doomsgoossens, H. Degreef, C. Holvoet, M. Maertens, Contact Derm. 3 (1977) 304–308.
[55] R.B. Croteau, E.M. Davis, K.L. Ringer, M.R. Wildung, Naturwissenschaften 92 (2005) 562–577.
[56] M.S. Baliga, S. Rao, J. Canc. Res. Ther. 6 (2010) 255–262.
[57] T.V. Kozyreva, V.P. Kozaruk, E.Y. Tkachenko, G.M. Khramova, J. Therm. Biol. 35 (2010) 428–434.
[58] D.D. McKemy, W.M. Neuhausser, D. Julius, Nature 416 (2002) 52–58.
[59] A.M. Peier, A. Moqrich, A.C. Hergarden, A.J. Reeve, D.A. Andersson, G.M. Story, Cell 108 (2002) 705–715.
[60] D.A. Andersson, H.W.N. Chase, S. Bevan, J. Neurosci. 24 (2004) 5364–5369.
[61] G. Turner, J. Gershenzon, E.E. Nielson, J.E. Froehlich, R.; Croteau, Plant Physiol. 120 (1999) 879–886.
[62] S.S. Mahmoud, M. Williams, R. Croteau, Phytochemistry 65 (2004) 547–554.
[63] R.B. Kjonaas, K.V. Venkatachalam, R. Croteau, Arch. Biochem. Biophys. 238 (1985) 49–60.
[64] K.L. Ringer, E.M. Davis, R. Croteau, Plant Physiol. 137 (2005) 863–872.
[65] M. Wildung, R. Croteau, Transgenic Res. 14 (2005) 365–372.
[66] S.S. Mahmoud, R. Croteau, Proc. Natl. Acad. Sci. 98 (2001) 8915–8920.
[67] A. Aharoni, M.A. Jongsma, H.J. Bouwmeester, Trends Plant Sci. 10 (2005) 594–602.
[68] S.S. Mahmoud, R. Croteau, Proc. Natl. Acad. Sci. 100 (2003) 14,481–14,486.
[69] J. Caissard, O. Faure, F. Jullien, M. Colson, A. Perrin, Plant Cell Rep. 16 (1996) 67–70.
[70] S. Dhawan, A. Shasany, A. Naqvi, S. Kumar, S. Khanuja, Plant Cell Tissue Organ Cult. 75 (2003) 87–94.
[71] A. Shasany, S. Khanuja, S. Dhawan, S. Kumar, J. Biosci. 25 (2000) 263–266.
[72] D. Johnson, T. Cummings, Plant Dis. 84 (2000) 235–238.
[73] M. Mucciarelli, S. Scannerini, C. Bertea, M. Maffei, New Phytol. 158 (2003) 579–591.
[74] M.A. van Agtmael, T.A. Eggelte, C.J. van Boxtel, Trends Pharmacol. Sci. 20 (1999) 199–205.
[75] E. Hsu, Trans. R Soc. Trop. Med. Hyg. 100 (2006) 505–508.
[76] G.D. Brown, Molecules 15 (2010) 7603–7698.
[77] K. Dost, G. Davidson, Analyst 128 (2003) 1037–1042.
[78] B. Engels, P. Dahm, S. Jennewein, Metab. Eng. 10 (2008) 201–206.
[79] R. Haynes, Curr. Top. Med. Chem. 6 (2006) 509–537.
[80] M.K. Jung, R.F. Schinazi, Bioorg. Med. Chem. Lett. 4 (1994) 931–934.
[81] D. De Clercq, J. Vercruysse, P. Verle, F. Niasse, A. Kongs, M. Diop, Trans. R Soc. Trop. Med. Hyg. 94 (2000) 90–91.
[82] Y. Zhang, K.H. Teoh, D.W. Reed, L. Maes, A. Goossens, D.J.H. Olson, J. Biol. Chem. 283 (2008) 21,501–21,508.

[83] M.E. Olsson, L.M. Olofsson, A. Lindahl, A. Lundgren, M. Brodelius, P.E. Brodelius, Phytochemistry 70 (2009) 1123–1128.
[84] C.M. Bertea, J.R. Freije, H. van der Woude, F.W.A. Verstappen, L. Perk, V. Marquez, Planta. Med. 71 (2005) 40–47.
[85] R.S. Sangwan, K. Agarwal, R. Luthra, R.S. Thakur, N. Singhsangwan, Phytochemistry 34 (1993) 1301–1302.
[86] M.S.R. Nair, D.V. Basile, Indian J. Chem. Sect. B-Org. Chem. Incl. Med. Chem. 31 (1992) 880–882.
[87] T.E. Wallaart, H.J. Bouwmeester, J. Hille, L. Poppinga, N.C.A. Maijers, Planta. 212 (2001) 460–465.
[88] L.K. Sy, N.Y. Zhu, G.D. Brown, Tetrahedron 57 (2001) 8495–8510.
[89] A. Ryden, C. Ruyter-Spira, W.J. Quax, H. Osada, T. Muranaka, O. Kayser, Planta. Med. 76 (2010) 1778–1783.
[90] L.K. Sy, G.D. Brown, Tetrahedron 58 (2002) 897–908.
[91] K.S. Ngo, G.D. Brown, Tetrahedron 55 (1999) 15,109–15,126.
[92] X. Cao, Z. Zong, X. Ju, Y. Sun, C. Dai, Q. Liu, Mol. Biol. Rep. 37 (2010) 1559–1567.
[93] K.H. Teoh, D.R. Polichuk, D.W. Reed, G. Nowak, P.S. Covello, FEBS Lett. 580 (2006) 1411–1416.
[94] T. Nafis, M. Akmal, M. Ram, P. Alam, S. Ahlawat, A. Mohd, Plant Biotechnol. Rep. 5 (2011) 53–60.
[95] W. Banyai, C. Kirdmanee, M. Mii, K. Supaibulwatana, Plant Cell Tissue Organ Cult. 67 (2010) 255–265.
[96] R. Yang, L. Feng, X. Yang, L. Yin, X. Xu, Q. Zeng, Planta. Med. 74 (2008) 1510–1516.
[97] J. Chappell, Plant Physiol. 107 (1995) 1–6.
[98] R. Yang, X. Zeng, Y. Lu, W. Lu, L. Feng, X. Yang, Planta. Med. 76 (2010) 734–742.
[99] G. Pu, D. Ma, J. Chen, L. Ma, H. Wang, G. Li, Plant Cell Rep. 28 (2009) 1127–1135.
[100] W. Banyai, M. Mii, K. Supaibulwatana, Plant Growth Regul. 63 (2011) 45–54.
[101] D. Ro, E. Paradise, M. Ouellet, K. Fisher, K. Newman, J. Ndungu, Nature 440 (2006) 940–943.
[102] Q. Zeng, F. Qiu, L. Yuan, Biotechnol. Lett. 30 (2008) 581–592.
[103] J.R. Anthony, L.C. Anthony, F. Nowroozi, G. Kwon, J.D. Newman, J.D. Keasling, Metab. Eng. 11 (2009) 13–19.
[104] X. Xu, J. Zhu, D. Huang, W. Zhou, Tetrahedron 42 (1986) 819–828.
[105] J. Yadav, R. Babu, G. Sabitha, Tetrahedron Lett. 44 (2003) 387–389.
[106] D. Toppmeyer, A.D. Seidman, M. Pollak, C. Russell, K. Tkaczuk, S. Verma, Clin. Cancer Res. 8 (2002) 670–678.
[107] M.C. Wani, H.L. Taylor, M.E. Wall, P. Coggon, A.T. Mcphail, J. Am. Chem. Soc. 93 (1971) 2325.
[108] R. Croteau, R.E.B. Ketchum, R.M. Long, R. Kaspera, M.R. Wildung, Phytochem. Rev. 5 (2006) 75–97.
[109] J. Wu, L. Lin, Appl. Microbiol. Biotechnol. 62 (2003) 151–155.
[110] D.C. Hao, G. Ge, P. Xiao, Y. Zhang, L. Yang, PLoS One 6 (2011) e21,220.
[111] S. Jennewein, R.M. Long, R.M. Williams, R. Croteau, Chem. Biol. 11 (2004) 379–387.
[112] A. Schoendorf, C.D. Rithner, R.M. Williams, R. Croteau, Proc. Natl. Acad. Sci. 98 (2001) 1501–1506.
[113] F. Guerittevoegelein, D. Guenard, P. Potier, J. Nat. Prod. 50 (1987) 9–18.
[114] G. Ge, H. Luan, Y. Zhang, Y. He, X. Liu, L. Yang, Rapid Commun. Mass Spectrom. 22 (2008) 2315–2323.
[115] K. Walker, R. Long, R. Croteau, Proc. Natl. Acad. Sci. 99 (2002) 9166–9171.

[116] M. Chau, S. Jennewein, K. Walker, R. Croteau, Chem. Biol. 11 (2004) 663–672.
[117] H.J. Hartzell, The Yew Tree: A Thousand Whispers. Hulgosi Books, 1991.
[118] G. Strobel, X. Yang, J. Sears, R. Kramer, R. Sidhu, W. Hess, Microbiology-(UK) 142 (1996) 435–440.
[119] Z. Hu, L.L. Ou, G.L. Zhang, Y.P. Yu, Chin. Chem. Lett. 19 (2008) 130–132.
[120] S. Yang, S. Lu, Y. Yuan, Biochim. Biophys. Acta Mol. Cell. Biol. Lipids 1781 (2008) 123–134.
[121] A. Khosroushahi, M. Valizadeh, A. Ghasempour, M. Khosrowshahli, H. Naghdibadi, M. Dadpour, Cell. Biol. Int. 30 (2006) 262–269.
[122] G. Francis, Z. Kerem, H.P.S. Makkar, K. Becker, Br. J. Nutr. 88 (2002) 587–605.
[123] K. Papadopoulou, R.E. Melton, M. Leggett, M.J. Daniels, A.E. Osbourn, Proc. Natl. Acad. Sci. 96 (1999) 12,923–12,928.
[124] C. Sun, Y. Li, Q. Wu, H. Luo, Y. Sun, J. Song, BMC Genomics 11 (2010) 262.
[125] M.H. Lee, J.H. Jeong, J.W. Seo, C.G. Shin, Y.S. Kim, J.G. In, D.C. Yang, J.S. Yi, Y.E. Choi, Plant Cell Physiol. 45 (2004) 976–984.
[126] Y.R. Pokharel, N.D. Kim, H. Han, W.K. Oh, K.W. Kang, Nutr. Cancer 62 (2010) 252–259.
[127] Y. Liang, S. Zhao, Plant Biol. 10 (2008) 415–421.
[128] E. Lambert, A. Faizal, D. Geelen, Appl. Biochem. Biotechnol. 2011.
[129] J. Vincken, L. Heng, A. de Groot, H. Gruppen, Phytochemistry 68 (2007) 275–297.
[130] C.M.M. Gachon, M. Langlois-Meurinne, P. Saindrenan, Trends Plant Sci. 10 (2005) 542–549.
[131] O.T. Kim, S.H. Kim, K. Ohyama, T. Muranaka, Y.E. Choi, H.Y. Lee, Plant Cell Rep. 29 (2010) 403–411.
[132] Y. Liang, S. Zhao, X. Zhang, Plant Mol. Biol. Rep. 27 (2009) 298–304.
[133] C.A. Howitt, B.J. Pogson, Plant Cell Environ. 29 (2006) 435–445.
[134] M. Aluru, Y. Xu, R. Guo, Z. Wang, S. Li, W. White, J. Exp. Bot. 59 (2008) 3551–3562.
[135] G.A. Armstrong, J. Bacteriol. 176 (1994) 4795–4802.
[136] C.K. Shewmaker, J.A. Sheehy, M. Daley, S. Colburn, D.Y. Ke, Plant J. 20 (1999) 401–412.
[137] I. Pott, D.E. Breithaupt, R. Carle, Phytochemistry 64 (2003) 825–829.
[138] V. Hagenimana, E.E. Carey, S.T. Gichuki, M.A. Oyunga, J.K. Imungi, Ecol. Food Nutr. 37 (1999) 455–473.
[139] N.K. Choudhury, R.K. Behera, Photosynthetica. 39 (2001) 481–488.
[140] F. Rivas, F. Fornes, M.J. Rodrigo, L. Zacarias, M. Agusti, Sci. Hortic. 127 (2011) 482–487.
[141] K.P. West, J. Nutr. 132 (2002) 2857S–2866S.
[142] H. Park, S.S. Kreunen, A.J. Cuttriss, D. DellaPenna, B.J. Pogson, Plant Cell 14 (2002) 321–332.
[143] X.D. Ye, S. Al-Babili, A. Kloti, J. Zhang, P. Lucca, P. Beyer, Science 287 (2000) 303–305.
[144] J.A. Paine, C.A. Shipton, S. Chaggar, R.M. Howells, M.J. Kennedy, G. Vernon, Nat. Biotechnol. 23 (2005) 482–487.

Chapter 6

Trypanocidal Monoterpenes: Lead Compounds to Design Future Trypanocidal Drugs

Soodabeh Saeidnia and Ahmad Reza Gohari
Medicinal Plants Research Center, Tehran University of Medical Sciences, Tehran, P.O. Box 14155 6451, Iran

INTRODUCTION

The protozoa *Trypanosoma* is responsible for two distinct diseases in humans. *Trypanosoma cruzi* causes American trypanosomiasis, also known as Chagas disease (in tropical Americas) and *Trypanosoma brucei* causes Human African trypanosomiasis, also known as sleeping sickness (in Africa). Chagas disease is caused by *T. cruzi* which is transmitted to humans *via* blood-sucking insects (vectors), named triatomine bugs.

Among the Americas, there are about 20 million infected people from Southern California to Argentina and Chile, most of them do not know they are infected. Infection is life-long without treatment. Chagas disease has an acute phase and a chronic latent stage that may result in heart or gastrointestinal disease. Indeterminate stage of Chagas disease follows the acute phase, when the infection remains silent for decades or life. These people are still infected with *T. cruzi* and are capable of transmitting it to others. Approximately 30% of infected people will develop chronic symptoms after a latency period of 10–30 years.

In people who have immunodeficiency (due to cancer, AIDS or chemotherapy), Chagas disease can reactivate. Chagasic cardiac disease is a result of persistent parasite presence in the heart which can cause abnormal heart rhythms and cardiomyopathy with symptoms of palpitations, syncope, heart failure, or sudden death. Gastrointestinal complications consist of enlarged oesophagus or colon [1,2].

Human African trypanosomiasis, well known as sleeping sickness, is a parasitic disease transmitted by the bite of the 'Glossina' insect, commonly known

Bioactive Natural Products (Part Q), Vol. 37. http://dx.doi.org/10.1016/B978-0-444-59514-0.00006-7

as the tsetse fly. There are two types of African trypanosomiasis, named for the regions in Africa in which they are found: East African trypanosomiasis and West African trypanosomiasis. East African trypanosomiasis is caused by the parasite *T. b. rhodesiense*. West African trypanosomiasis is caused by *T. b. gambiense*. The parasites are spread by tsetse flies, found only in Africa. Infection can rarely be communicated by blood transfusion or organ transplant. Exposure can occur at any time. People obtain the disorder from the bite of an infected tsetse fly. In rare cases, an infected pregnant woman can pass the infection to her baby. The disease affects mostly poor populations living in remote rural areas of Africa. If the illness remains untreated, it might be fatal. Travellers are another group at risk especially if they venture through regions where the insect is common. Usually, the disease is not found in urban areas, although some cases have been reported in suburban areas of Kinshasa, capital of the Democratic Republic of Congo and Luanda, the capital city of Angola [3].

In 1995, WHO (the World Health Organization) Expert Committee estimated that 60 million people were at risk with an estimated 300,000 new cases per year in Africa, with fewer than 30,000 cases diagnosed and treated. In 2009, the number of new reported cases fell down and WHO considered that it was due to increased control. So far, very little progress has been made in the development of new drugs against this disease. Most drugs, still in use, were developed one or more decades ago, and they are generally toxic and of limited effectiveness. The most recently introduced compound, eflornithine, is only useful against sleeping sickness caused by *T. b. gambiense*, and is prohibitively expensive for the African developing countries [4].

Treatments

In the early stage of Chagas disease, most anti-parasitic drugs are effective however acute Chagas disease often goes undetected. Anti-chagastic agents, nifurtimox or benznidazole, are currently recommended. All patients with acute Chagas disease, children with indeterminate-phase and those with reactivated infection should receive treatment. It has to be considered that anti-parasitic medications have significant side effects and offer no benefits in patients with established cardiac or gastrointestinal complications of chronic Chagas disease. Unfortunately, these drugs are only active against the acute phase of disease and present strong side effects. Also there are some problems with drugs in terms of long period of treatment, toxicity and even cost in endemic regions [5,6].

It is important to begin treatment as soon as possible in African trypanosomiasis. The type of drug treatment depends on the type and stage of African trypanosomiasis. Combination therapy may be more effective than monotherapy for the treatment of late-stage *T. b. gambiense* trypanosomiasis. Adverse effects are more common in patients who received eflornithine monotherapy. However, the nifurtimox–eflornithine combination is a promising regimen for use

in late-stage *T. b. gambiense* trypanosomiasis. Anti-parasitic agents are usually used *via intra-venus* injection (IV) in early stage of African trypanosomiasis. Suramin is a polysulfonated naphthylamine derivative of urea and a trypanocidal agent which works by inhibiting parasitic enzymes and growth factors [7]. Trivalent arsenical (Melarsoprol) used in the late or CNS stage of African trypanosomiasis is trypanocidal *via* inhibiting parasitic glycolysis. Unfortunately, it can be toxic and even fatal in 4–6% of the cases. Eflornithine is generally better tolerated and it is less toxic than arsenic drugs and available *via* the World Health Organization. Pentamidine isethionate is usually used for early (or stage 1) African trypanosomiasis and administered *via* IV (intravenous). This compound is strongly bounded to tissues, including spleen, liver, and kidney. It does not effectively penetrate the blood–brain barrier and, therefore, it is not used to treat CNS infection [8–10]. These reasons give a priority to discover new trypanocidal compounds.

Prevention

There are currently no vaccines available for treatment of trypanosomiasis. The development of an effective prophylactic vaccine faces many challenges. Research investigations in animal and human patients have revealed many aspects of the pathogenic mechanism of disease and targets of protective immunization. Various antigens, vehicles and adjuvants have been detected in animal models, and sometimes the efforts have been successful [11,12].

Cruzipain is one of the most important metabolites of the *T. cruzi* that has been widely studied in the recent decades. It is involved in parasite metabolism and identified as an important candidate for vaccine development and also trypanocidal drug design. This enzyme is a sulphated glycoprotein [13].

Natural Resources for Drug Discovery

Medicinal plants provided a broad range of effective components against parasites. There are many reports about the screening of herbal extracts and aromas against *T. cruzi*, the etiological agent of Chagas disease, and other strains. In the first stage, plant extracts have to be evaluated against epimastigotes of *Trypaosoma* sp., then on the bloodstream forms of the parasite (trypomastigotes), and finally by *in vivo* experiments, using animal model systems [14–16].

It is known from the literature that secondary plant metabolites have exhibited anti-trypanocidal activity. Most of the important compounds belong to flavonoids (catechin, epicatechin, gallocatechin, epigallocatechin and apigenin), chalcones, xanthones, dibenzofuranones, anthraquinones, aporphine alkaloids (actinodaphnine, cassythine and dicentrine), triterpenes (ursolic acid, oleanolic acid, colosolic acid), diterpenes (komaroviquinone and dracocephalone A), sesquiterpenes (zaluzanin D, dehydrocostus lactone and neurolenin B) and finally monoterpenes [17–21]. Although, various natural products have been

introduced as trypanocidal compounds, there is limited information about the mechanisms of action and further researches are required.

Monoterpenes

This group of compounds is widespread in plants, from algae to monocots and dicots, also found in fungi. Some are encountered in bacteria, but they are usually not accumulated in bacteria. They often produce the intense odour and one commonly present among essential oil components. They are usually accumulated in epithelial cells and living cavities in the plants and their production is associated with plastids in plant cells. They are rarely accumulated in tissue cultures and mostly synthesized in higher plants from the deoxy xylulose phosphate (DOXP) pathway. Geranyl pyrophosphate or diphosphate (GPP) is the key intermediate in the synthesis of most monoterpenes. In higher plants, the five-carbon building blocks of all terpenoids, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAP), are derived from two independent pathways localized in different cellular compartments. The methyl erythritol phosphate (MEP or nonmevalonate) pathway, localized in the plastids, is thought to provide IPP and DMAP for hemiterpene, monoterpene, and diterpene biosynthesis, whereas the cytosol-localized mevalonate pathway provides C5 units for sesquiterpene biosynthesis. Incorporation of glycosides into monoterpenes occurs more readily than for usual monoterpenes. These glycosides work better for biosynthetic studies. So far, at least 1000 monoterpenes are known. They belong to more than 38 major structural types [22–24].

The first acyclic monoterpene to be formed is geraniol and others (including linalool, nerol, and citronellol) are produced from it *via* enzymatic conversion. Many common monocyclic monoterpenes can be formally derived from α-terpineol as a key intermediate, and it is likely that limonene, terpinolene and 1,8-cineole are derived from it. Monoterpenoids often has strong smell and colourless oily appearance with boiling point around 140–180°C, although a few (such as camphor) are crystalline. They are usually isolated and identified by gas chromatography/mass spectroscopy. Also thin layer chromatography is a simple way for primary detection [25].

Monoterpenes are effective on expression of Ras (belongs to a large family of proteins known as low-molecular weight G-proteins and promotes cell division when a growth factor is present on the cell surface) and Ras-related proteins, which play key roles in signal transduction pathways and are known to regulate diverse cellular functions, in the absence and presence of mevalonate depletion. The ability of monoterpenes to regulate the expression of the Ras-related proteins was found to be independent of effects on cell proliferation and total cellular protein synthesis or degradation [26]. Many monoterpenes have been described as potent inhibitors of seed germination and growth of several plant species. Geraniol and carvone significantly inhibited the germination

of garden cress, at the highest concentration tested [27]. Several halogenated monoterpenes, isolated from the red alga *Plocamium cartilagineum*, have been reported to show cytotoxic effects against colon and cervical adenocarcinoma cells [28]. Literature also shows that monoterpenes are emitted by forests and form aerosols that can serve as cloud condensation nuclei (CCN) and increase the brightness of clouds then cool the climate [29].

Besides many biological activities of monoterpenes, they have also been reported to be the potent trypanocidal compounds in some *in vitro* and *in vivo* systems. Therefore, in this review we focussed on the trypanocidal activity of various types of monoterpenes which might be a guide to find the lead compounds and finally active drugs in treatment of both American and African trypanosomiasis. A review on the related literature revealed that monoterpenes would appear to act through multiple mechanisms in the chemotherapy of trypanosomiasis. While, there is no report about the classification of the active monoterpenes (in relation to their activity) against various *Trypanosoma* intermediates such as epimastigotes, trypomastigotes or amastigotes. Occasionally, this classification was not related to monoterpene skeleton types which have been often explained in the phytochemical papers and books. In these cases, we had to divide them according to their main functional groups such as monoterpene hydroperoxides.

Trypanocidal Assay Approaches

Chemotherapy against human trypanosomiasis relies on limited number of drugs that might cause severe side effects. Because trypanosomiasis is a disease of poor people especially in Latin America and Africa, the traditional pathways to drug development are not available. ATP-bioluminescence assay could be used to rapidly and efficiently screen compound libraries against trypanosomes in a high throughput-screening format [30]. Most of the papers reported the screening of plant extracts and fractions *via in vitro* assay against epimastigotes or trypomastigote forms of *T. cruzi* [31,32]. In order to do drug screening for this neglected disease, recently a validated cell-based and high throughput assay is reported, which simultaneously measures efficacy against the intracellular amastigote stage and toxicity to the host cells [33].

The recent modern approaches for target-directed drug discovery include the identification of suitable enzymes involving in metabolic pathways of *T. cruzi*, which can be the target in the development of novel drugs with higher efficacy and lower toxicity. The bloodstream form of *T. cruzi* has no functional tricarboxylic acid cycle, and is highly dependent on glycolysis for ATP production. The glycolytic enzymes are attractive targets for trypanocidal drug design because *T. cruzi* shows a great dependence on glycolysis as a source of energy. One of these proteins, glycossomal glyceraldehyde-3-phosphate dehydrogenase (GAPDH), catalyzes the oxidative phosphorylation of glyceraldehyde-3-phosphate (GAP) to 1,3-bisphosphoglycerate (BPG) [34].

Trypanocidal Monoterpenes

Limonene Derivatives

Limonene (**1**) is an important monoterpene and a major compound of the volatile oils of *Citrus* plants, available in both enantiomeric forms: (+)-*R*- and (−)-*S*-limonene, Fig. 1. Limonene is a building block for the organic synthesis of several compounds as well as a chiral auxiliary in asymmetric syntheses [35,36]. Literature shows that essential oils consisting limonene can present antimicrobial, anti-fungal, antimalarial and anti-tumoral activities [37].

Monoterpenes are widely distributed in nature and could be the suitable precursor substrates for production of valuable natural fragrance compounds. Among various monoterpenes, limonene as a substrate of microbial transformation or chemical semi synthesis has been a source of many oxy functional derivatives. (*R*)-(+)-Limonene can be obtained from citrus peel oil in annual amounts of up to 50 million kg as a byproduct. Therefore, limonene is a cheap and simply available starting material to transform into other valuable flavour compounds. Graebin *et al*. (2010) have recently reported the evaluation of some semisynthetic limonene derivatives against *in vitro* cultures of *T. cruzi* epimastigotes (Tulahuen 2 strain) [37]. Their results showed that, compounds **2** (IC_{50} = 19.2μM) and **2′** (IC_{50} = 29.5μM) were effective against *T. cruzi* compared with nifurtimox (**3**) as a positive control (IC_{50} = 7.7μM). In a structural point of view, chemical changes in the parent compound limonene (IC_{50} > 50μM) were conducted by active compound (**2**), Fig. 1.

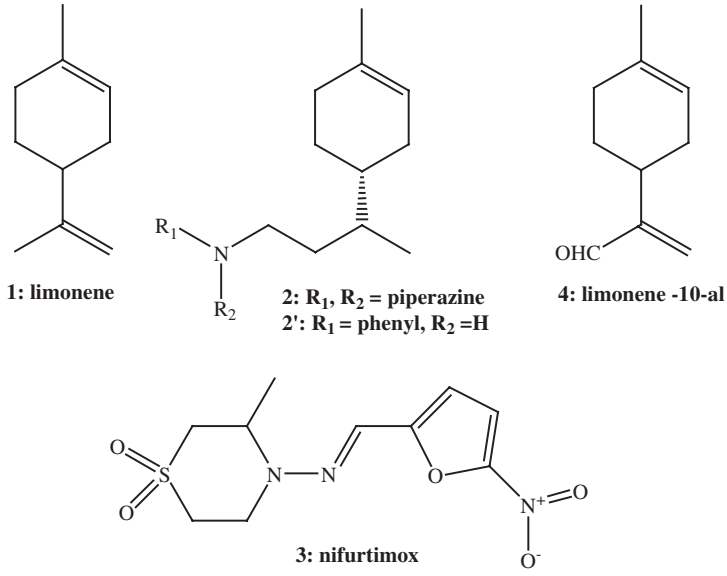


FIGURE 1 Structures of the trypanocidal limonene derivatives and nifurtimox.

The isolation and trypanocidal investigation of limonene-10-al (**4**) a monoterpene aldehyde, from the ethyl acetate fraction and the essential oil of *Dracocephalum kotschyi* and *Dracocephalum subcapitatum* has been previously reported, Fig. 1 [17,38,39]. This aldehyde indicated a potent activity (MLC = 3.1μM). Other alcoholic metabolites of limonene, such as *p*-mentha-8-en-1, 2-diol, limonene-10-ol and its glucosides (Limonen-10-ol 10-O-β-D-glucopyranoside, Limonen-10-ol 10-O-β-D-glucopyranosyl-β-D-glucopyranoside), did not show trypanocidal activity even at 400μM [38].

Linear Monoterpenes

It is reported that the monoterpene aldehyde, geranial (**5**) and neral (**6**), isolated from ethyl acetate extracts of *D. kotschyi* and *D. subcapitatum* as well as Trypanocidal activities (determined as MLC, minimum lethal concentrations) of the isolated both geranial and neral against epimastigotes of *T. cruzi* were reported as 3.1μM [38].

It is reported that the presence of geranial and neral, as a well-known mixture named citral, could be responsible for activity of the essential oil of *Nepeta cataria* against *T. cruzi* [40]. Although geranial and neral (the aldehydes) were reported as effective agents against *T. cruzi*, their relative alcohols, geraniol (**7**) and nerol (**8**), have been evaluated against *T. brucei brucei* bloodstream forms and geraniol reported as a weakly active compound (IC_{50} = 95μM) while, nerol was inactive, Fig. 2 [41]. The compound, geranyl acetone (**9**) proved to have an IC_{50} towards the *T. brucei* to 50μM. The IC_{50} value of compound **9**

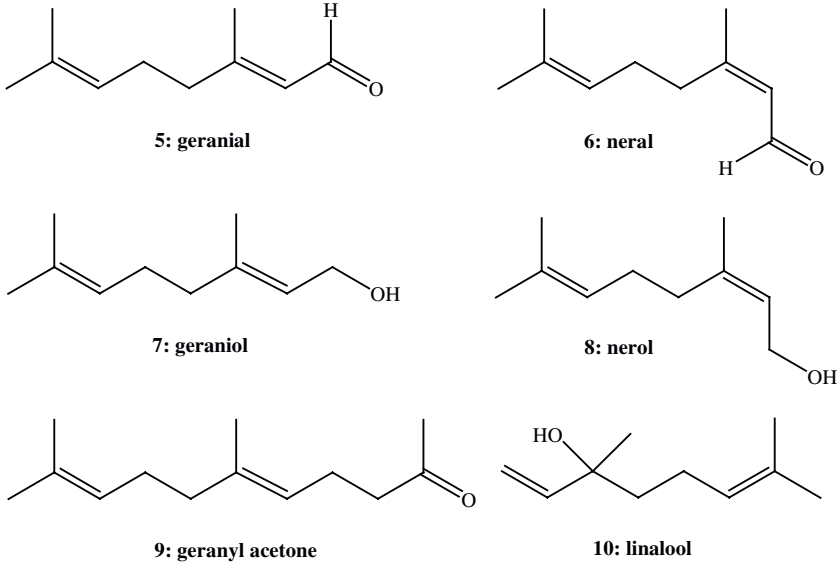


FIGURE 2 Structures of the trypanocidal linear monoterpenes.

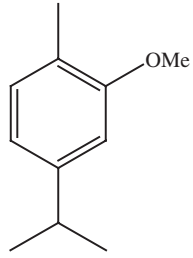
obtained on mammalian cells was superior to 500μM which had selectivity indices superior to 19.

There are few reports around another trypanocidal linear monoterpene, linalool (**10**), but it showed no considerable activity. We discuss more about that under miscellaneous monoterpenes.

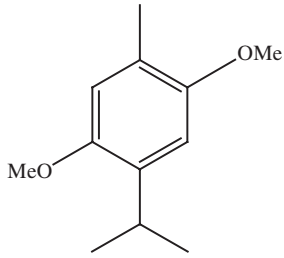
Para-cymene Derivatives

In 1991, Hocquemiller *et al*. reported the *in vitro* trypanocidal evaluation (against 20 strains of *T. cruzi*) of four aromatic monoterpenes, O-methyl carvacrol (**11**), thymoquinol methyl ether (**12**), espintanol (**13**) and O-methyl espintanol (**14**), Fig. 3, isolated from the petroleum ether fraction of *Oxandra espintana* (Annonaceae). Among them only espintanol indicated the trypanocidal activity (IC_{90} = 25μM). The values for other monoterpenes were higher than 100(μg/ml) [42].

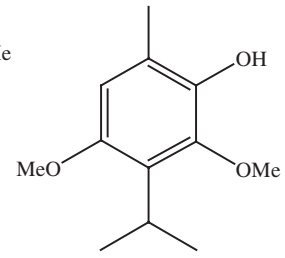
In 2007, we reported the isolation of thymol (**15**) and carvacrol (**16**), Fig. 3, two phenolic monoterpenes, from the essential oil of *Satureja macrantha*. These compounds were tested biologically against the epimastigotes of *T. cruzi* and represented a moderate activity [43]. These simple volatile components have been previously reported for their antibacterial synergism [44]. Recently, biological activities of thymol and carvacrol have been tested against free and



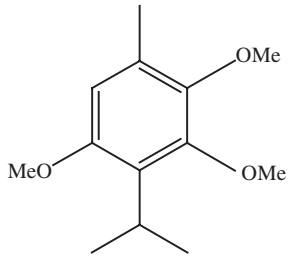
11: O -metyl carvacrol



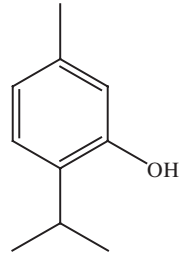
12: thymoquinol methyl ether



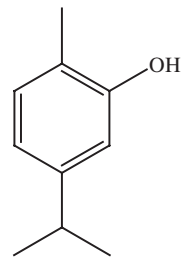
13: espintanol



14: O-methyl espintanol



15: thymol



16: carvacrol

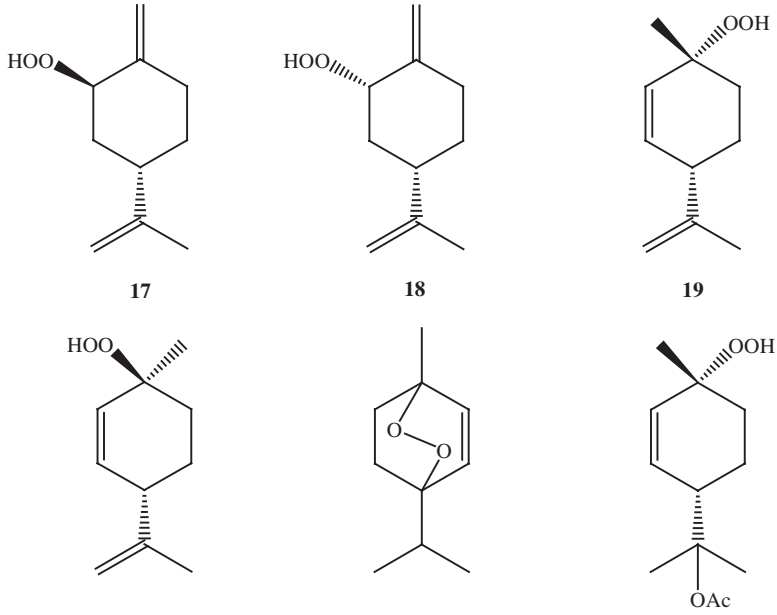
FIGURE 3 Structures of the trypanocidal p-cymene derivatives.

intracellular forms of *T. cruzi* parasites as well as Vero and THP-1 mammalian cell lines [45]. In the *T. cruzi* as says, thymol and carvacrol were active against epimastigotes. Thymol (IC_{50} = 3.6, IC_{90} > 30μM, SI (Selectivity Index) = 11.8) was active on intracellular amastigotes of *T. cruzi* infected Vero cells. Thymol is the main constituent of the essential oils of many *Lippia* spp., originated from Colombia. Carvacrol (IC_{50} = 27.3μM, SI = 11.8) was moderately effective against amastigote forms. In this assay, *p*-cymene itself, found in the essential oils of *Lippia* spp., was reported to be weakly active against epimastigotes of *T. cruzi* (IC_{50} = 28μM, SI = 4.1) but not against amastigote forms (IC_{50} = 190μM, SI = 0.6).

Monoterpene Hydroperoxides

Kiuchi *et al*., in 2002, reported the isolation of four monoterpene hydroperoxides, (−)-(2*S*,4*S*) (**17**) and (−)-(2*R*,4*S*)-*p*-mentha-1(7),8-dien-2-hydroperoxides (**18**) and (−)-(1*R*,4*S*) (**19**) and (−)-(1*S*,4*S*)-*p*-mentha-2,8-dien-1-hydroperoxides (**20**) along with ascaridol (**21**), Fig. 4, from the ethyl acetate extract of the fresh aerial parts of *Chenopodium ambrosioides* (Chenopodiaceae).

The hydroperoxides showed stronger trypanocidal activity (MLCs = 1.2, 1.6, 3.1 and 0.8μg/ml, respectively) than ascaridol (MLC = 23μg/ml). These compounds were also tested with the HeLa cell infection assay and were effective at 10μg/ml bloodstream forms. At 1μg/ml, the latest hydroperoxide completely



17 **18** **19**

20 **21: ascaridol** **22**

FIGURE 4 Structures of the trypanocidal monoterpene hydroperoxides.

inhibited the infection of HeLa cells by the trypomastigotes but they did not inhibit the proliferation of amastigotes in the infected cells. The *p*-menthane alcohols, detected in the essential oil of this plant, because of hydroperoxides degradation during steam distillation, did not show trypanocidal activity even in higher concentrations (>400μg/ml) [46,47].

Another monoterpene hydroperoxide (1*R*,4*S*)-1-hydroepoxy-*p*-menth-2-en-8-ol acetate (**22**) has been isolated from *Laurus nobilis* (Lauraceae) and showed trypanocidal activity against epimastigotes of *T. cruzi* (MLC = 1.4μM). It is reported that this compound, Fig. 4, could inhibit the infection of trypomastigotes to Hela cells (98%) and also proliferation of amastigotes in Hela cells (83%) [48].

Pinenes and p-Menthans

Mikus *et al*. (2000) reported the toxicity effect of the essential oils of *Melissa officinalis, Thymus vulgaris* and *Melaleuca alternifolia* to bloodstream forms of *T. brucei* more than to human HL-60 cells [49]. Also they evaluated the toxicity of eight terpenes which were detected in those essential oils. Among the tested compounds, the ED_{50} against *T. brucei* for α- (**23**) and β-pinenes (**24**), sabinene (**25**), terpinen-4-ol (**26**) and terpinolene (**27**) were 4.1, 54.8, 17.7, 0.02 and 31.0 (μM) respectively, Fig. 5. Therefore, terpinen-4-ol showed a potent anti-trypanosoma effect and its selectivity index (ratio of cytotoxicity on human HL-60/anti-trypanosomal activity) was 1025, comparable with commercially available drugs such as suramin (**28**), Fig. 6.

Other Monoterpenes

Trypanocidal activity of *Syzygium aromaticum, Ocimum basilicum* and *Achillea millefolium* essential oils and some of their constituents, eugenol (**29**) and linalool (**10**) was reported by Santoro *et al*. [50], on *T. cruzi* epimastigote and bloodstream trypomastigote forms, Fig. 7. Their investigation showed that the values obtained for epimastigotes treated with eugenol and linalool were 246 and 162.5mM, respectively, while treatment of bloodstream trypomastigotes resulted in IC_{50} (24h) values of 76 and 264 (mM) for eugenol and linalool, respectively [50].

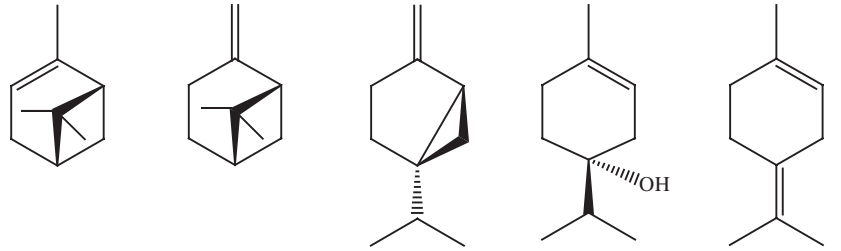
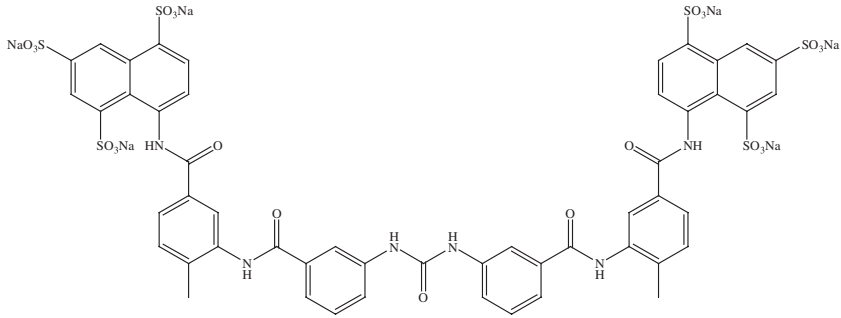


FIGURE 5 Structures of the trypanocidal monoterpenes belonging to pinene and *p*-menthan skeletons.



28: suramin

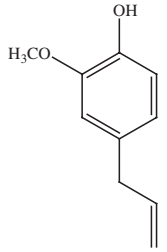
FIGURE 6 Structure of suramin, a trypanocidal medicine, used as a positive control.

Eugenol is a phenyl propanoid and the main constituent of *S. aromaticum* essential oil, and it is also an important chemical constituent of essential oils from many aromatic plants, such as *Dicipelium cariophyllatum*, *Pimenta dioica*, *Croton zehntneri* var. *eugenoliferum*, and *C. zehntneri* [51]. Linalool is an alcoholic monoterpene and the main component of *O. basilicum* oil (46.97%). It has been shown that linalool-rich essential oils extracted from several plants have antimicrobial properties but because of the little effect of linalool alone on *T. cruzi*, this monoterpene did not candidate for further investigation [52].

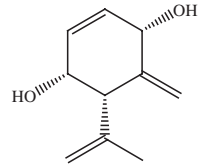
Piquerol A (**30**), a monoterpene, has been isolated from *Piquera trinervia* Cav., Fig. 7, and found to be a biologically active substance in several *in vitro* studies. Piquerol A consists of tetra-substituted cyclohexene ring which possesses three non-conjugated double bonds. Bioassay with epimastigotes of *T. cruzi* showed the *in vitro* trypanosomal activity of piquerol A, which was related to its concentration. This compound arrested the growth of epimastigotes of *T. cruzi* at the concentration of more than 200(μg/ml) which is similar to the reported concentration of ofloxacin, a patented 4-quinolonecarboxylic acid derivative [53]. There is no report about the trypanocidal activity of piquerol B, the stereoisomer of piquerol A, against *T. cruzi* or *T. brucei*. However, both of them have been reported to indicate acaricidal activity on *Boophilus microplus* [54].

Nibret *et al*. (2010) reported the chemical compositions, trypanocidal, and cytotoxic activities of the essential oils extracted from three Ethiopian medicinal plants, *Hagenia abyssinica* (Rosaceae), *Leonotis ocymifolia* (Lamiaceae), and *Moringa stenopetala* (Moringaceae). The trypanocidal activity against *T. brucei* and antileukaemic (HL-60) effects of the three essential oils was studied. Citronellal (**31**), a linear monoterpene aldehyde, was evaluated against *T. brucei* and showed a moderate activity ($IC_{50} = 13.5$μg/ml) but it also showed cytotoxicity on human cells and was not a good candidate for more investigations (SI = 10.5) [55].

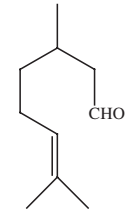
The oil of *M. stenopetala* seeds and its main compound, benzyl isothiocyanate (**32**) showed the most potent trypanocidal activities (IC_{50}, 5.03μg/ml



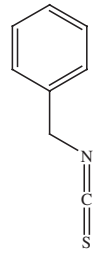
29:eugenol



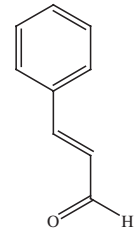
30: piquerol A



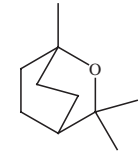
31: citronellal



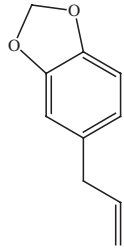
32:benzyl isothiocyanate



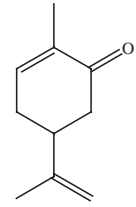
33:cinnamaldehyde



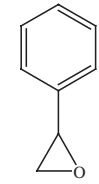
34:1,8-cineole



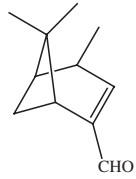
35:safrole



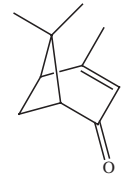
36:carvone



37:styrene oxide

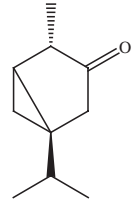


38:myrtenal

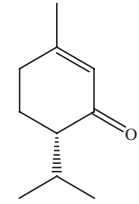


39:verbenone

40:camphor



41:thujone



42:piperitone

FIGURE 7 Structures of some trypanocidal compounds. The compounds **30**, **34**, **36** and **38–42** are monoterpenes.

and 1.20μg/ml, respectively). Twenty-eight compounds of the essential oils were also studied for their structure–activity relationships using trypanosomes and human leukaemia cells. Cinnamaldehyde (IC_{50}=2.93μg/ml) (**33**), 1,8-cineole (IC_{50}=83.15μg/ml) (**34**), safrole (IC_{50}=18.40μg/ml) (**35**), carvone (IC_{50}=12.94μg/ml) (**36**), styrene oxide (IC_{50}=3.76μg/ml) (**37**), carvacrol (IC_{50}=11.25μg/ml) (**16**) together with some sesquiterpenes showed the most potent trypanocidal activities. Among the components tested, **34** and **36** are monoterpenes and others are the simple volatile constituents especially belonging to phenyl propanoids (compounds, **29, 33, 35** and **37**) and consisting of isothiocyanate, carboxylic acid and epoxide functional groups. The compounds, carvone (SI=17.46) and styrene oxide (SI=19.92) showed good selective indices for the parasite with minimal toxicity on the human leukaemia cells. These compounds could therefore serve as lead structures for the development of trypanocidal agents with higher potency, Fig. 7 [55].

STRUCTURE–ACTIVITY RELATIONSHIPS FOR TRYPANOCIDAL MONOTERPENES

A literature review revealed that secondary metabolites with aldehyde functional group usually showed the most potent trypanocidal activity against both *T. cruzi* and *T. brucei*. These are not only from monoterpene compounds but also from other volatile compound classes which were studied for their structure–activity relationships. Among the aldehydes, limonene-10-al (**4**), geanial (**5**) and neral (**6**) showed the best trypanocidal activity against *T. cruzi* and cinnamaldehyde (**33**) was the best against *T. brucei* followed by citronellal (**31**) and myrtenal (**38**). All of them except citronellal have a C–C double bond conjugated with the carbonyl group, which is well known as α,β-unsaturated aldehydes. An additional aromatic group further makes cinnamaldehyde the most active compound from others [55]. Unfortunately, the trypanocidal and cytotoxic activities of aldehydes showed correlation with their chemical structures. Among the mentioned aldehyde, citral (geranial and neral) was studied more on metacyclogenesis of *T. cruzi* and could effectively block the *in vitro* metacyclogenesis. Citral also killed the resulting metacyclic trypomastigote form, which may have occurred through alterations of membrane permeability. It makes citral a good inhibitory drug candidate for more studies on the *T. cruzi* metacyclogenesis process [56]. About the mechanism of action for α,β-unsaturated aldehydes, it is reported that they are able to form covalent bonds with amino residues of proteins and inactivate most of the proteins that are likely to affect numerous cellular activities [57]. In trypanosomes, they may form aldehyde-thiol adducts with sulphur containing components. Therefore, decrease of buffering agents can create oxidative stress in cells. The same mechanism of action is suggested in human cell lines [55]. About the *p*-cymene derivatives, phenolic compounds showed better activity than *p*-cymene itself. The isomers thymol (**15**) and carvacrol (**16**) showed different trypanocidal

activities against *T. cruzi*. Their cytotoxic activities against human HL-60 cells were approximately the same. However, thymol showed the best activity against epimastigotes and intracellular amastigotes of *T. cruzi* compared with other phenols. Carvacrol (**16**) and espintanol (**13**) indicated the same activity. It is reported that carvacrol was more active against *T. brucei* than thymol [55]. In addition, it is found to be more active against Hep-2 cell than thymol [58]. It seems that changing hydroxyl position on the benzene ring makes different susceptibility on *T. cruzi* and *T. brucei*. Furthermore, lower steric hindrance of phenolic hydroxyl in carvacrol may not be the only reason for such a different activity.

Among the various types of monoterpenes, the class that contained hydroperoxides showed strong trypanocidal activity against *T. cruzi*. When these compounds were reduced to alcoholic structures they did not show any activity. For this reason, the functional hydrogen peroxide group should be responsible for trypanocidal activity. As shown in the literature, hydroperoxy group oxidates the glutathione, pyruvic and alpha-ketoglutaric acids and the oxidative decarboxylation of pyruvic acid makes them toxic [59]. On the other hand, the compounds that contain hydroperoxide functional group are easily destroyed due to alcohol for example during hydro-distillation of the essential oils [46].

Limonene itself is not a noteworthy trypanocidal compound but it can be used as a parent compound to produce (*via* semi synthetical or biochemical process) the products which contain aldehyde (**4**), piperazine (**2**), phenyl (**2'**), hydroperoxides (**17–22**) or ketone (**36**) and show more potent trypanocidal activity against *T. cruzi*. The hydrocarbon limonene also exhibited substantial trypanocidal activity against *T. brucei* ($IC_{50} = 11.25\mu g/ml$, SI = 4.5) [55]. As shown in the literature, limonene is a lipophilic hydrocarbon consisting of exocyclic and terminal methylene unit which can interact strongly with SH of proteins. This makes limonene toxic against trypanosoma as well as HL-60 cells [60].

Among the pinenes, α-pinene (**23**) showed more potent activity against *T. brucei* than β-pinene (**24**). This compound is a lipophilic monoterpene such as limonene but has an endocyclic methylene just apposite of limonene. At this point, it is worth mentioning that terminal exocyclic methylene makes the hydrocarbonic monoterpenes very strong. By the way, moderate trypanocidal activity was observed from sabinene (**25**), as a hydrocarbonic monoterpene with an exocyclic methylene.

Bibliography reveals that although many alcoholic monoterpenes, belonging to all classes, have been evaluated against both African and American trypanosomes, most of them were inactive or weakly active. For example, *p*-mentha-8-en-1, 2-diol, limonene-10-ol and the alcoholic forms of monoterpene hydroperoxides are all inactive. There is an exception, terpinen-4-ol (**26**), which showed a potent anti-trypanosoma (*T. brucei*) effect with a good selectivity index is comparable with trypanocidal drugs of choice such as suramin.

Terpinen-4-ol is likely to be a mediator for *in vivo* and *in vitro* activity of tea tree oil (*M. alternifolia*) against azole-susceptible and azole-resistant *Candida albicans* [61].

Among the miscellaneous monoterpenes, carvone (**36**) showed a good trypanocidal activity. Its trypanocidal activity was reported to be more than verbenone (**39**), camphor (**40**), thujone (**41**), and piperitone (**42**), Fig. 7, which showed low activity between 37 and 41μM [55]. Carvone contains two double bonds and isopropenyl side chain on cyclic ring. These differences with others may be responsible for the good trypanocidal effect of carvone. As previously mentioned the terminal methylene of carvone should be very reactive with SH of cysteine residues of proteins and affects the normal activity of cells [60]. These compounds were cytotoxic against the human cell (HL-60). Carvone, as the main constituent of *Carum carvi, Anethum graveolen* and *Mentha spicata*, has been reported to show various biological activities with minimal *in vitro* cytotoxic effect (IC_{50} = 0.9μM) against human cell (Hep-2) [58] and showed no adverse effects on rats even at the dose of 2500ppm for 1 year [62]. These points together with the edible sources of carvone make it a promising drug of future for therapy of African trypanosomiasis.

Eugenol (**29**), styrene oxide (**37**), safrole (**35**) and cinnamaldehyde (**33**) are derivatives of phenyl propanoid compounds which formed *via* different biochemical pathway, well known as shikimate pathway, while monoterpenes are produced *via* acetate pathway. Among these compounds, styrene oxide contains epoxides and showed the potent trypanocidal activity. The epoxide ring can form covalent bonds with the amino and SH groups in proteins or secondary basic nitrogen of DNA bases [60]. It seems that the benzene ring in these compounds especially when connected to epoxide in styrene oxide enhances their trypanocidal activity. Safrole is a bicyclic volatile compound consisting of a benzene ring fused to a dioxy ring of five-carbon and allylic side chain. Safrole is currently classified as a carcinogen category 2 and mutagen category 3 in the IFRA-IOFI labelling manual 2009. Out of the three alleged human carcinogens, safrole, estragole and methyl eugenol, safrole is arguably the weakest. The hazardous dose of *Sassafras officinale* oil for humans (which typically contains 80% safrole) has been determined as 0.66mg/kg, based on experimental animal data [63].

Many of the isolated volatile components from the essential oils of the plants have been reported to show trypanocidal activities. These compounds, bearing different functional groups, mostly belong to monoterpenes. The current review summarized the present information until 2010 on trypanocidal activities of various classes of monoterpene compounds in order to better design and develop novel drugs with higher efficacy and lower toxicity. This paper suggests the scientists and researchers to select an appropriate compound for more investigation, for example, to promote the mechanisms of action, cell uptake of drugs, enhancement of the biological activities of the compounds which serve as trypanocidal drugs for treatment of trypanosomiasis.

CONCLUSION

Monoterpenes have been known for centuries as the fragrant components of the essential oils obtained from several parts of the plants. These compounds presented numerous actions, such as allelochemical functions between plants, and between plants and predators or in wound healing. Many monoterpenes possess antitumour activity in animal and cell models. They have also antioxidant, antimicrobial and anti-protozoa properties. Literature review shows that there are some papers which reported anti-trypanosoma activity of monoterpenes. Some of these active compounds build up *p*-menthan and *p*-cymene skeletons and others contain hydroperoxides, aldehydes, acids or alcohol moieties. Among the compounds tested and reported, terpinen-4-ol showed a potent anti-trypanosoma (*T. brucei*) effect with a good selectivity index comparable with trypanocidal drug, suramin. Carvone is another candidate for more anti *T. brucei* investigations. Monoterpenes bearing aldehyde functional group generally are potent trypanocidal compounds against both *T. cruzi* and *T. brucei* but need more structural changes for reduction of human cell toxicity. Importance of monoterpenes, due to the potent volatile compounds, simple structure, easy synthesis, and cost effective, make them drug of choice for the future therapy of trypanosomiasis especially in the form of systemic absorption inhalers or aromatherapy.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge grants for research promotion from the Ministry of Health and Medical Education of Iran and Tehran University of Medical Sciences, Health Services Grants. Also, we are indebted to Miss Najmeh Mokhber-Dezfuli for her assistance in the preparation of the manuscript.

ABBREVIATIONS

BPG	1,3-bisphosphoglycerate
DMAP	dimethylallyl pirophosphate
DOXP	deoxy xylulose phosphate
GAP	glyceraldehyde-3-phosphate
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GPP	geranyl pyrophosphate
IFRA	International Fragrance Association
IOFI	International Organization of the Flavor Industry
IPP	isopentenyl diphosphate
MEP	methyl erythritol phosphate
MIC	minimum inhibitory concentration
SI	selectivity index
T. cruzi	*Ttypanosoma cruzi*
T. brucei	*Trypanosoma brucei*
T. b. gambiense	*Trypanosoma brucei gambiense*

REFERENCES

[1] J.C.P. Dias, A.C. Silveira, C.J. Schofield, Mem. Inst. Oswaldo Cruz. 97 (2002) 603–612.
[2] J.R. Coura, S.L. De Castro, Mem. Inst. Oswaldo Cruz. 97 (2002) 3–24.
[3] P.P. Simarro, G. Cecchi, M. Paone, J.R. Franco, A. Diarra, J.A. Ruiz, E.M. Fevre, F. Courtin, R.C. Mattioli, J.G. Jannin, Int. J. Health Geog. 9 (2010) 1–18.
[4] R. Docampo, S.N. Moreno, Parasitol. Res. 90 (2003) 10–13.
[5] H. Cerecetto, M. Gonzalez, Curr. Top. Med. Chem. 2 (2002) 1187–1213.
[6] N. Garg, V. Bhatia, Expert Rev. Vaccines 4 (2005) 867–880.
[7] M. Brigotti, R.R. Alfieri, P.G. Petronini, D. Carnicelli, Biochimie. 88 (2006) 497–503.
[8] C. Schmid, M. Richer, C.M. Bilenge, T. Josenando, F. Chappuis, C.R. Manthelot, J. Infect. Dis. 191 (2005) 1922–1931.
[9] J. Pepin, F. Milord, C. Guern, Lancet. 1 (1989) 1246–1250.
[10] R. Brun, O. Balmer, Curr. Opin. Infect. Dis. 19 (2006) 415–420.
[11] G.A.M. Cross, Int. J. Parasitol. 31 (2001) 427–433.
[12] A. Harder, G. Greif, A. Haberkorn, Parasitol. Res. 87 (2001) 778–780.
[13] V.G. Duschak, A.S. Couto, Curr. Med. Chem. 16 (2009) 3174–3202.
[14] A.R. Gohari, S. Saeidnia, A. Hadjiakhoondi, A. Naghinejad, T. Yagura, J. Med. Plants 7 (2008) 44–48.
[15] A.R.P. Ambrozin, P.C. Vieira, J.B. Fernandes, M.F.G. Fernandes da silva, S. De Albuquerque, Mem. Inst. Oswaldo Cruz. 99 (2004) 227–231.
[16] S. Sepulveda-Boza, B.K. Cassels, Planta. Med. 62 (1996) 98–105.
[17] S. Saeidnia, A.R. Gohari, M. Ito, F. Kiuchi, G. Honda, Z. Naturforsch. 60 (2005) 22–24.
[18] A.R. Gohari, S., Saeidnia, K. Matsuo, N. Uchiyama, T. Yagura, M. Ito, G. Honda, J. Nat. Med. 57 (2003) 250–252.
[19] N. Uchiyama, M. Ito, F. Kiuchi, G. Honda, Y. Takeda, O.K. Khodzhimatov, O.A. Ashurmetov, Tetrahedron Lett. 45 (2004) 531–533.
[20] D. Tasdemir, M. Kaiser, R. Bruner, V. Yardley, T.J. Schmidt, F. Tosun, P. Ruedi, Antimicrobial. Agents Chemother. 50 (2006) 1352–1364.
[21] J.D. Maya, B.K. Cassels, P. Iturriaga-Vasquez, J. Ferreira, M. Faundez, N. Galanti, A. Ferreira, A. Morrello, Comp. Biochem. Physiol. 146 (2007) 601–620.
[22] P.M. Dewick, Medicinal Natural Products, A Biosynthetic Approach. John Wiley & Sons Ltd, UK, 2009.
[23] E. Pichersky, D.R. Gang, Trends Plant Sci. 205 (2000) 439–445.
[24] N. Dudareva, S. Andersson, I. Orlova, N. Gatto, M. Reichelt, D. Rhodes, W. Boland, J. Gershenzon, Proc. Natl. Acad. Sci. USA 102 (2005) 933–938.
[25] J.B. Harborne, H. Baxter, G.P. Moss, Phytochemical Dictionary: A Handbook of Bioactive Compounds from Plants. Taylor and Francis, 2009.
[26] S.A. Holstein, R.J. Hohl, J. Lipid Res. 44 (2003) 1209–1215.
[27] L.D. Martino, E. Mancini, L.F.R. Almeida, V.D. Feo, Molecules 15 (2010) 6630–6637.
[28] C. De Ines, V.H. Argandona, J. Rovirosa, A. San-Martin, A.R. Diaz-Marrero, M. Cueto, A. Gonzalez-Coloma, Z. Naturforsch. 59c (2004) 339–344.
[29] D.V. Spracklen, B. Bonn, K.S. Carslaw, Phil. Trans. R. Soc. A 366 (2008) 4613–4626.
[30] Z.B. Mackey, A.M. Baca, J.P. Mallari, B. Apsel, A. Shelat, E.J. Hansell, P.K. Chiang, B. Wolff, K.R. Guy, J. Williams, J.H. McKerrow, Chem. Biol. Drug Des. 67 (2006) 355–363.
[31] C.F.F. Grael, W. Vichnewski, G.E.P. De Souza, J.L.C. Lopes, S. Albuquerque, W.R. Cunha, Phytother. Res. 14 (2000) 203–206.

[32] N. Uchiyama, F. Kiuchi, M. Ito, G. Honda, Y. Takeda, O.K. Khodzhimatov, O.A. Ashurmetov, J. Nat. Prod. 66 (2003) 128–131.
[33] J.C. Engel, K.K.H. Ang, S. Chen, M.R. Arkin, J.H. McKerrow, P.S. Doyle, Antimicrobial. Agents Chemother. 54 (2010) 3326–3334.
[34] P.C. Vieira, J. Mafezoli, M.T. Pupo, J.B. Fernandes, M.F.G.F. Da Silva, J. De Albuquerque, G. Oliva, F. Pavao, Pure Appl. Chem. 73 (2001) 617–622.
[35] J.P. Davidson, E.J. Corey, J. Am. Chem. Soc. 125 (2003) 13486–13489.
[36] P.A. Wender, L. Zhang, Org. Lett. 2 (2000) 2323–2326.
[37] C.S. Graebin, M.F. Madeira, J.K.U. Yokoyama-Yasunaka, D.C. Miguel, S.R.B. Uliana, D. Benitez, Eur. J. Med. Chem. 45 (2010) 1524–1528.
[38] S. Saeidnia, A.R. Gohari, N. Uchiyama, M. Ito, G. Honda, F. Kiuchi, Chem. Pharm. Bull. 52 (2004) 1249–1250.
[39] S. Saeidnia, A.R. Gohari, A. Hadjiakhoondi, A. Shafiee, Z. Naturforsch. 62 (2007) 793–796.
[40] S. Saeidnia, A.R. Gohari, A. Hadjiakhoondi, J. Med. Plants 1 (2008) 27–30.
[41] S. Hoet, C. Stevigny, M.F. Herent, J. Quetin-Leclercq, Planta. Med. 72 (2006) 480–482.
[42] R. Hocquemiller, D. Cortes, G.J. Arango, S.H. Myint, A. Cave, J. Nat. Prod. 54 (1991) 445–452.
[43] S. Saeidnia, A.R. Gohari, A. Hadjiakhoondi, Int. J. Essent. Oil Ther. 1 (2007) 184–186.
[44] S. Seungwon, K. Ji-Hyun, Planta. Med. 70 (2004) 1090–1092.
[45] P. Escobar, S.M. Leal, L.V. Herrera, J.R. Martinez, E. Stashenko, Mem. Inst. Oswaldo Cruz. 105 (2010) 184–190.
[46] F. Kiuchi, Y. Itano, N. Uchiyama, G. Honda, A. Tsubouchi, J. Nakajima-Shimada, T. Aoki, J. Nat. Prod. 65 (2002) 509–512.
[47] A. Ahmed, J. Nat. Prod. 63 (2000) 989–991.
[48] N. Uchiyama, K. Matsunaga, F. Kiuchi, G. Honda, A. Tsunouchi, J. Nakajima-Shimada, T. Aoki, Chem. Pharm. Bull. 50 (2002) 1514–1516.
[49] J. Mikus, M. Harkenthal, D. Steverding, J. Reichling, Planta. Med. 66 (2000) 366–368.
[50] G.F. Santoro, M.G. Cardoso, L.G.L. Guimaraes, L.Z. Mendonca, M.J. Soares, Exp. Parasitol. 116 (2007) 283–290.
[51] M. De Vincenzi, M. Silano, P. Stacchini, B. Scazzocchio, Fitoterapia. 71 (2000) 216–221.
[52] G. Mazzanti, L. Battinelli, G. Salvatore, Flav. Frag. J. 13 (1998) 289–294.
[53] C. Castro, M. Jimenez, M. Gonzalez-De La Parra, Planta. Med. 58 (1992) 281–282.
[54] M. Gonzalez-de la Parra, D. Chavez-Pena, M. Jimenez-Estrada, C. Ramos-Mundo, Pestic. Sci. 33 (1991) 73–80.
[55] E. Nibret, M. Wink, Phytomedicine 17 (2010) 911–920.
[56] J. Cardoso, M.J. Soares, Mem. Inst. Oswaldo Cruz. 105 (2010) 1026–1032.
[57] G. Witz, Free Radic. Biol. Med. 7 (1989) 333–349.
[58] A. Stammati, P. Bonsi, F. Zucco, R. Moezelaar, H.L. Alakomi, A. Von-Wright, Food Chem. Toxicol. 37 (1999) 813–823.
[59] A. Mucchielli, L. Saint-Lebe, C. R. Acad. Sci. Hebd. Seances. Acad. Sci. D. 283 (1976) 435–438.
[60] M. Wink, Curr. Drug. Metab. 9 (2008) 996–1009.
[61] F. Mondello, F. De Bernardis, A. Girolamo, A. Cassone, G. Salvatore, BMC Infect. Dis. 6 (2006) 158.
[62] E.C. Hagan, W.H. Hansen, O.G. Fitzhugh, P.M. Jenner, W.I. Jones, J.M. Taylor, E.L. Long, A.A. Nelson, J.B. Brouwer, Food Cosmet. Toxicol. 5 (1967) 141–157.
[63] N. Bisset, Herbal Drugs and Phytopharmaceuticals. CRC Press, Stuttgart, Germany, 1994 pp. 455–456.

Chapter 7

Plant and Fungi 3,4-Dihydroisocoumarins: Structures, Biological Activity, and Taxonomic Relationships

Alessandra Braca[†], Ammar Bader[*] and Nunziatina De Tommasi[‡]

[†]*Dipartimento di Scienze Farmaceutiche, Università di Pisa, Via Bonanno 33, Pisa, Italy*

[*]*Department of Pharmacognosy, Faculty of Pharmacy, Umm Al-Qura University, P.O. Box 13174, Makkah, Saudi Arabia*

[‡]*Dipartimento di Scienze Farmaceutiche e Biomediche,Università di Salerno, Via Ponte Don Melillo, Fisciano, Salerno, Italy*

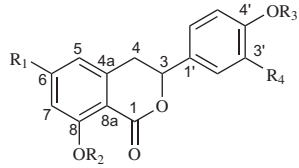
INTRODUCTION

Isocoumarin is the common name for 1*H*-2-benzopyran-1-one skeleton; they are derivatives of isochromene and all the natural isocoumarins bear oxygen atom at one or more of the six available positions. These oxygen atoms may be in the form of phenolic, ethereal or glycosidic functionalities groups. Particularly, 3,4-dihydroisocoumarins (DHICs) are characterized as saturated analogues between C-3/C-4. DHIC are a small group of interesting secondary metabolites produced by some plants, fungi, insects, and other living organisms. The structures of natural DHIC show various types of substitution in their basic skeleton including *O*-glycosylation and methylation and this variability influences deeply their biological activities.

The first scientific isolation report on DHIC is dating back to 1916 with the isolation of hydrangenol (**1**), (Fig. 1) from the flowers of *Hydrangea hortensia* Smith (Saxifragaceae) [1]; later in the same year another DHIC, named phyllodulcin (**2**) (Fig. 1), was isolated from the flowers of *Hydrangea macrophylla* Seringe *var. thunbergii* Makino [2]. Compound **1** is one of the most common DHIC in the plant kingdom.

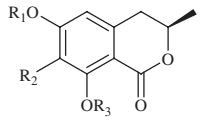
Phyllodulcin, which has a very sweet taste, 400–800 times sweeter than sucrose, became later a target of intensive studies to develop safe and effective

Bioactive Natural Products (Part Q), Vol. 37. http://dx.doi.org/10.1016/B978-0-444-59514-0.00007-9



1 R_1=R_2=R_3=R_4=H
2 R_1=R_2=R_4=H R_3=Me
9 R_1=R_3=R_4 =H R_2=glc
14 R_1=OH R_2=R_3=R_4 =H
15 R_1=R_4=OH R_2=R_3 =H
17 R_1=R_2=R_3=H R_4 =*O*-glc
24 R_1=R_2=R_4 =H R_3=glc
44 R_1=R_3=R_4 =H R_2=Me
50 R_1=OMe R_2=R_3=R_4=H

FIGURE 1



3 R_1=Me R_2=R_3=H
4 R_1=Me R_2=OMe R_3=H
5 R_1=R_3=H R_2=OMe
6 R_1=R_2=R_3=H
56 R_1=Me R_2=H R_3=glc

FIGURE 2

sweeteners. Besides the sweet derivative of DHIC, some other compounds such as 6-methoxymellein (**3**) (Fig. 2), found in stressed carrots, has attracted the attention for its bitter taste.

The plants containing DHIC are not systematically related: e.g., *Aloe* sp. (Xanthorrhoeaceae), *Scorzonera* sp., *Crassocephalum* sp. and *Tragopogon* sp. (Asteraceae), *Xyris* sp. (Xyridaceae), *Daucus* sp. and *Notopterygium* sp. (Apiaceae), *Hydrangea* sp. (Hydrangeaceae), *Polygala* sp. (Polygalaceae), *Montrouziera* sp. and *Hypericum* sp. (Guttiferae), *Kigelia* sp. (Bignoniaceae), and *Haloxylon* sp. (Chenopodiaceae). Also fungi are an important source of DHIC.

DHICs exhibit important biological and pharmacological properties such as antimicrobial, anticancer, plant growth inhibition, insecticidal, antimalarial, anticancer, antioxidant, and antiallergic activities. Besides the beneficial effects of DHIC, some of them have been investigated for their high toxicity and for their allergenic properties.

This review deals with the most important findings of the last 30 years in the field of DHIC found in a wide number of organisms including plants, fungi, liverworts, and insects, and it will show the importance of this class of secondary

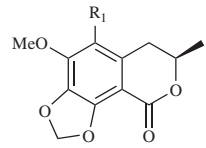
metabolites in the prevention and treatment of some diseases as well as their importance in food as new lead of promising class of sweeteners.

PLANTS

After the isolation of hydrangenol (**1**) and phyllodulcin (**2**) (Fig. 1), a significant number of DHIC have been isolated and characterized from plant species. 6-Methoxymellein (**3**) and kigelin (**4**) (Fig. 2) were isolated from the roots of *Kigelia pinnata* DC. (syn. *Kigelia africana* Benth., Bignoniaceae), a smell spreading tree with pendulous racemes of dull liver-coloured flowers and a long-stalked large gourd-like fruit. Compounds **4** and 3-demethylkigelin (**5**) were identified also from the plant bark [3,4]. Compound **4** and its congeners have close biogenetic and structural similarities to the ochratoxins, reticulol, canescin, and 6-hydroxyramulosin, the chlorine-containing metabolites from *Sporormia affinis* Sacc. (Sporormiaceae).

(+)-6-Hydroxymellein (**6**) (Fig. 2) was obtained from the flowers of *Cassia siamea* Lam. (Caesalpiniaceae), one of the commonest trees of India [5]. Compound **6** was first isolated from a mutant of *Aspergillus terreus* (Trichocomaceae) and then from *Daucus carota* L. (Apiaceae) roots stored under stressed condition and from *Pyricularia oryzae* Cavara (Magnaporthaceae). Two structurally related DHIC, dihydroinversin or 3*R*-methyl-6-methoxy-7, 8-methylenedioxydihydroisocoumarin (**7**) and 3*R*-methyl-5,6-dimethoxy-7, 8-methylenedioxydihydroisocoumarin (**8**) were reported from the New Zealand liverwort, *Wettsteinia schusterana* Grolle (Adelantaceae) (Fig. 3) [6]. This is the only report about the presence of DHIC in liverworts.

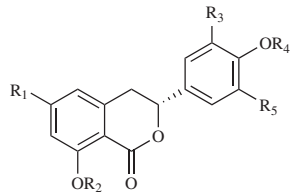
Hydrangea species (Saxifragaceae), plants cultivated as ornamental in pots and garden, are rich in DHIC. *H. macrophylla* var. *macrophylla* produces, together with **1**, its 8-*O*-β-glucoside (**9**) (Fig. 1). *H. macrophylla* Seringe var. *thunbergii* Makino whose leaves crumpled, fermentated, and dried constituted Hydrangeae Dulcis Folium, a rare natural medicine indigenous to Japan, where it is called Amacha, is extensively used in confectionery, drinks, and foods. The leaves methanolic extract of *H. macrophylla* Seringe var. *thunbergii* Makino, used in Japan after fermentation and darkening as a tea in Hanamatsuri Celebration (Birth of Buddha) [7], afforded (3*R*)-phyllodulcin



7 R_1=H
8 R_1=OMe

FIGURE 3

(**10**), 3*R*- and 3*S*-phyllodulcin 3'-*O*-glucosides (**11**) and (**12**), 3*S*-phyllodulcin 8-*O*-β-glucoside (**13**), thunberginols C (**14**), D (**15**), and E (**16**), thunberginol G 3'-*O*-β-glucoside (**17**), 3*R*- and 3*S*-thunberginol H 8-*O*-glucosides (**18**) and (**19**), 3*R*- and 3*S*-thunberginol I 4'-*O*-glucosides (**20**) and (**21**), 3*R*- and 3*S*-thunberginol I 8-*O*-glucosides (**22**) and (**23**), hydrangenol 4'-*O*-β-glucoside (**24**), 3*R*- and 3*S*-hydrangenol 4'-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranosides (**25**) and (**26**) (Figs. 1, 4, and 5) [8–10]. The 3-phenyl-substituted DHICs having the 4'-hydroxyl group are known to show tautomer-like behaviour at C-3 and this type of DHIC existed as a racemic mixture; on the other hand DHIC having the 4'-methoxyl group are known to be stable.

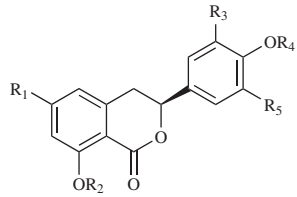


10 R_1=R_2=R_3=R_5=H R_4=Me
11 R_1=R_2=R_5=H R_4=Me R_3=Oglc
16 R_1=OH R_2=R_3=R_5=H R_4=Me
18 R_1=R_5=H R_4=Me R_3=OMe R_2=Oglc
20 R_1=R_2=R_5=H R_3=OMe R_4=glc
22 R_1=R_4=R_5=H R_3=OMe R_2=Oglc
25 R_1=R_2=R_3=R_5=H
28 R_1=R_4=H R_2=glc R_3=R_5=Me

FIGURE 4

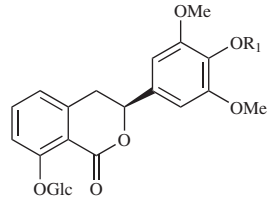
Finally, from *H. macrophylla* subsp. *serrata* (Thumb.) Makino macrophyllosides A (**27**), B (**28**), and C (**29**) were isolated [11] (Figs. 4 and 6).

DHICs appear also to be a useful chemotaxonomic marker within the genus *Aloe* (Xanthorrhoeaceae). Feralolide (**30**) (Fig. 7), characterized as (3*R*)-3,4-dihydro-6,8-dihydroxy-3-(2'-acetyl-3',5'-dihydroxyphenyl)methyl-1*H*-[2]benzopyran-1-one was isolated from a commercial sample of Cape aloe (dried exudates of *Aloe ferox* Miller or, occasionally, its hybrids with *Aloe africana* Miller or *Aloe spicata* Baker) where it occurs in 0.01% yield [12], while its 3'-*O*-β-D-glucopyranosyl derivative (**31**) (Fig. 7) was reported in 1994 from the leaf exudates of *Aloe hildebrandtii* Baker [13]. The latter compound was found to be fairly widespread in the genus *Aloe*: the proportion of this compound with respect to the total leaf phenolics varied from 1 to 15% (in *A. hildebrandtii* was found to be 4%). In recent years, 3,4-dihydro-6,8-dihydroxy-3-(2'-acetyl-3'-hydroxy-5'-methoxyphenyl)methyl-1*H*-[2]benzopyran-1-one (**32**), the 5'-methyl derivative of feralolide, was isolated from powdered sap (Fig. 7). Its structure was unambiguously established with the help of X-ray crystal structure analysis [14,15].



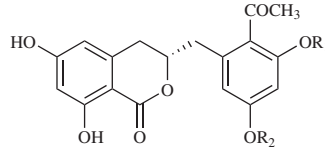
12 R_1=R_2=R_5=H R_4=Me R_3=Oglc
13 R_1=R_2=R_5 =H R_2=glc R_4=Me
19 R_1=R_5=H R_4=Me R_3=OMe R_2=Oglc
21 R_1=R_2=R_5=H R_3=OMe R_4=glc
23 R_1=R_4=R_5=H R_3=OMe R_2=Oglc
26 R_1=R_2=R_3=R_5=H R_4=api-(1-6)-glc
41 R_1=OH R_2=R_3=R_5=H R_4=Me
42 R_1=OH R_2=glc R_3=R_5=H R_4=Me
43 R_1=OH R_2=rha-(1-6)-glc R_3=R_5=H R_4=Me
45 R_1=R_3=R_5=H R_2=Me R_4=glc
46 R_1=R_2=R_3=R_5=H R_4=rha-(1-6)-glc
47 R_1=OMe R_2=glc R_3=R_5=H R_4=Me
51 R_1=OMe R_2=R_3=R_4=R_5=H

FIGURE 5



27 R_1=Me
29 R_1=H

FIGURE 6

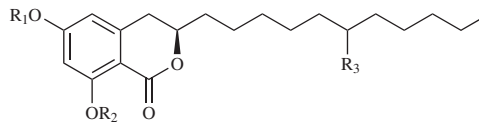


30 R_1=R_2=H
31 R_1=glc R_2=H
32 R_1=H R_2=CH_3

FIGURE 7

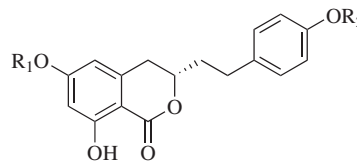
The genus *Ononis* (Fabaceae, Trifolieae tribe) is known to produce DHIC. From the methanol-soluble fraction of the *n*-hexane extract of *Ononis natrix* L., a perennial herb distributed throughout Jordan, (3*R*)-8-hydroxy-6-methoxy-3-undecyl-3,4-dihydroisocoumarin (**33**), (3*R*)-6,8-dihydroxy-3-undecyl-3,4-dihydroisocoumarin (**34**), (3*R*)-6,8-dihydroxy-3-(6-oxoundecyl)-3,4-dihydroisocoumarin (**35**), and (3*R*)-6,8-dihydroxy-3-(6-hydroxyundecyl)-3,4-dihydroisocoumarin (**36**) were isolated (Fig. 8) [16,17]. Compounds **33**, **34**, and **36** were also identified in *O. natrix* subsp. *hispanica*, a plant that grows in coastal zones of the Iberian Peninsula [18]. The synthesis of the principal DHIC of *O. natrix* was reported by Rama *et al*. in 1993 [19], while Hussain *et al*. performed a detailed study on their EIMS mass fragmentation pattern [20].

Agrimonolide (**37**) (Fig. 9) was reported as a component of *Agrimonia pilosa* Ledeb. (Rosaceae) roots since 1958 [21]. *A. pilosa* is a perennial herb distributed throughout South Korea and its roots have been used as haemostatic, antimalarial, and antidysenteric remedies in oriental medicine. In 2004, Park *et al*. isolated also its glycoside, agrimonolide 6-*O*-β-D-glucopyranoside (**38**) (Fig. 9) [22]. This latter compound was also identified together with desmethylagrimonolide 6-*O*-β-D-glucopyranoside (**39**) (Fig. 9) in the plant aerial parts [23]. Compound **37** was characterized in the ethanolic extract of *Spiraea formosana* Hayata (Rosaceae) stems [24], while its glycoside **38** was obtained from the leaves of *Lawsonia inermis* L. (Lythraceae) [25].



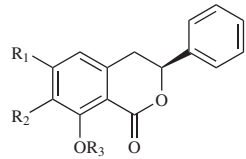
33 R_2=R_3=H R_1=Me
34 R_1=R_2=R_3=H
35 R_1=R_2=H R_3= =O
36 R_1=R_2=H R_3=OH

FIGURE 8



37 R_1=H R_2=CH_3
38 R_1=glc R_2=CH_3
39 R_1=glc R_2=H

FIGURE 9

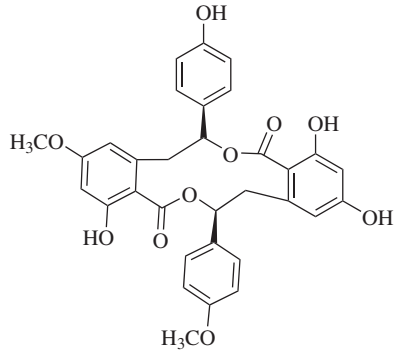


40 R_1=OH R_2=R_3=H
61 R_1=OH R_2= OMe R_3=H
62 R_1=R_2=O-CH_2-O R_3=Me

FIGURE 10

Montroumarin (**40**) (Fig. 10) was synthesized by Sakai *et al*. (1974) [26]. In 2000 Ito *et al*. reported its isolation from *Montrouziera sphaeroidea* Pancher Ex Planchon et Triana (Guttiferae) [27]. Successively, Lu *et al*. in 2007 reported the occurrence of **40** in the aerial parts of *Dioscorea nipponica* (Dioscoreaceae) [28].

The genus *Scorzonera* (Asteraceae, Lactuceae tribe, Scorzonerinae subtribe) includes several European species distributed all over the continent, from northern Russia to Spain, Crete, and Jordan. Chemical investigation of this genus yielded DHIC such as scorzocreticin (**41**), scorzocreticoside I or 8-*O*-β-D-glucopyranosylscorzocreticin (**42**), scorzocreticoside II or 8-*O*-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl]scorzocreticin (**43**) (Fig. 5) obtained from *Scorzonera cretica* Willd. [29]; compounds **1**, **24**, (±)-scorzotomentosin (**44**) (Fig. 1), and (−)-scorzotomentosin 4'-*O*-β-glucoside (**45**) (Fig. 2) isolated from *Scorzonera tomentosa* L. [30]; compounds **1** (Fig. 1) and **45** (Fig. 5) purified from *Scorzonera latifolia* (Fisch. & Mey.) DC. [31]; compounds **1**, **9**, **24**, **26**, **44**, and 3*S*-hydrangenol 4'-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside (**46**) (Figs. 1 and 5) identified recently from *Scorzonera judaica* [32], "Jordanian Viper's Grass" a perennial herb that grows in the desert and dry places of Jordan and used as food by Bedouins. Compounds **1**, **9**, **24**, **26**, **44**, and **46** were obtained from the $CHCl_3$–MeOH (9:1) extract of the plant roots and were elucidated by mass spectrometry, 1D- and 2D-NMR, and CD spectroscopy. Compound **42** was isolated together with its 6-*O*-methyl derivative, 6-*O*-methylscorzocreticoside (**47**) (Fig. 5) also from *Tragopogon porrifolius* L. subsp. *porrifolius* (Asteraceae, Lactuceae tribe, Scorzonerinae subtribe), highlighting the close phylogenetic relationship of the genera *Scorzonera* and *Tragopogon* [33]. A recent phytochemical re-investigation of *T. porrifolius* yielded the dimeric DHIC tragoponol (**48**) (Fig. 11), constituted by the open-chained forms of two different monomethoxylated DHIC moieties, scorzocreticin and hongkongenin, which are connected *via* two ester bonds to form a macrolide with two lactone moieties featuring a 12-membered ring [34]. Compound **48** is the first natural compound featuring a 7,8,15,16-tetrahydrodibenzo[c,i][1,7]dioxacyclododecine-5,13-dione ring system.



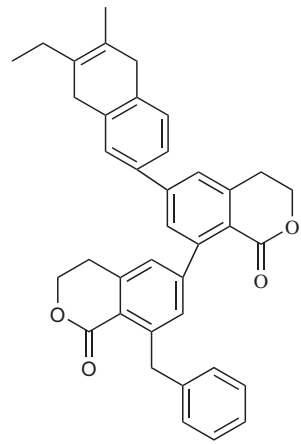
48

FIGURE 11

A dimeric DHIC, salvadorin (**49**), (Fig. 12) was isolated from the whole plant methanolic extract of *Salvadora oleoides* Decne (syn. *Salvadora stocksii* Wight) (Salvadoraceae), a small tree found in the arid regions of Pakistan and Western India [35].

The rhizomes of *Notopterygium forbesii* Boiss (Apiaceae) have been used for the treatment of cold, rheumatism, and ache in Chinese folk medicine. Chemical investigation of the constituents of plant rhizomes EtOH extract led to the isolation and characterization of 6-methoxyhydrangenol (**50**) (Fig. 1) [36].

Wu *et al*. in 2007 reported the isolation and structural elucidation of hongkogenin (**51**) (Fig. 5) from *Polygala hongkongensis* Hemsl (Polygalaceae). This is the only report on the occurrence of DHIC in the *Polygala* genus [37].



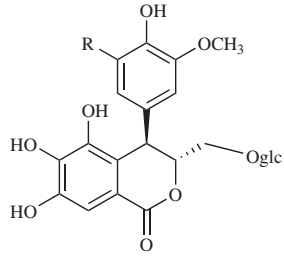
49

FIGURE 12

Investigation of the methanol extract stem bark of *Caryocar glabrum* (Aubl.) Pers. (Caryocaraceae) led to the isolation of two DHIC glucosides, members of a new biogenetic pathway for the isocoumarins nucleus involving shikimate derived A-ring (gallic acid) coupled with a phenyl propanoid derivative. These compounds were elucidated as (3*S*,4*S*)-3-(β-D-glucopyranosyloxymethyl)-3,4-dihydro-5,6,7-trihydroxy-4-(4'-hydroxy-3'-methoxyphenyl)-1*H*-[2]-benzopyran-1-one (**52**) and (3*S*,4*S*)-3-(β-D-glucopyranosyloxymethyl)-3,4-dihydro-5,6,7-trihydroxy-4-(4'-hydroxy-3',5'-dimethoxyphenyl)-1*H*-[2]-benzopyran-1-one (**53**) (Fig. 13) [38].

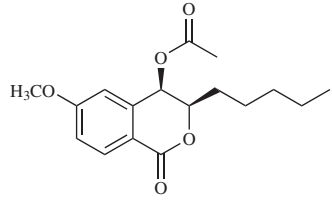
As a part of a phytochemical study directed towards the isolation of biologically active compounds from *Xyris* species, Guimarães *et al*. in 2008 obtained from *Xyris pterygoblephara* Steud. (Xyridaceae), the 3,4-disubstituted DHIC (3*R*,4*R*)-(−)-6-methoxy-3,4-dihydro-4-acethoxy-5-*n*-penthyl-1H-2-benzopyran-1-one (**54**) (Fig. 14) [39]. This is the only report on the isolation of a DHIC from a Xyridaceae species, although isocoumarins have been described from *X. indica* L. The presence of DHIC substituted either at C-3 and C-4 is uncommon in plant kingdom while they occur in fungi.

In the course of chemical investigation on the constituents of the Chinese mangrove associate *Catunaregam spinosa* (Thunb.) Tirveng (Rubiaceae), 3-(2-hydroxypropyl)-8-hydroxyl-3,4-dihydroisocoumarin (**55**) (Fig. 15) was characterized. The stereochemistry at the two chiral centres remained undetermined [40].



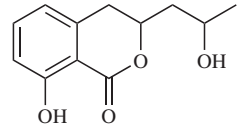
52 R=H
53 R=OMe

FIGURE 13



54

FIGURE 14



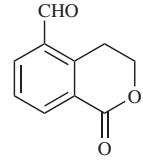
55

FIGURE 15

6-Methyl-6-methoxy-3,4-dihydroisocoumarin-8-*O*-β-D-glucopyranoside (**56**) (Fig. 2) was obtained from the alcoholic extract of *Saxifraga montana* H. (Saxifragaceae) whole plant, a perennial herbaceous species widely distributed in the northwest of China. This compound was also identified through TLC in cultured carrot cells [41].

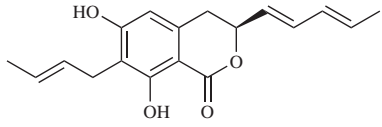
Erythrocentaurin or 5-formyl-2,3-dihydroisocoumarin (**57**) (Fig. 16) was isolated from *Centaurium erythraea* Rafn. (Gentianaceae) aerial parts [42]. The compound was also identified in the essential oil of the plant, although it was one of the minor constituents (0.1%) [43].

Bioassay-guided fractionation of the $CHCl_3$-soluble extract of the stem bark of *Crassocephalum biafrae* S. Moore (Asteraceae) resulted in the isolation of three DHIC, named biafraecoumarins A (**58**), B (**59**), and C (**60**) (Fig. 17). The plant, together with other *Crassocephalum* species, is widely used as food additive and in traditional medicine in many African countries [44].

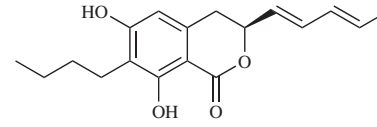


57

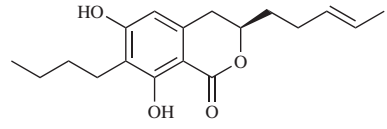
FIGURE 16



58



59



60

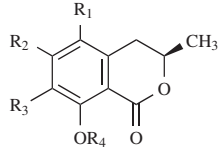
FIGURE 17

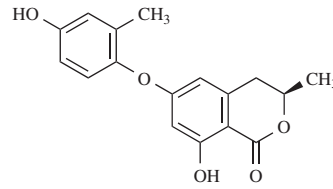
Nedialkov *et al*. in 2007 reported the isolation and structural elucidation of annulatomarin (**61**) (Fig. 10) from the aerial parts of *Hypericum annulatum* Moris (Guttiferae), a herbaceous plant growing on the Balkan Peninsula [45]. The synthesis of 6-*O*-methylannulatomarin and 7-demethylannulatomarin was described in 2010 [46].

(*S*)-4-Methoxy-7-phenyl-7,8-dihydro[1,3]dioxolo[4,5-*g*]isochromen-5-one (**62**) (Fig. 10) was isolated from a dichloromethane extract of *Haloxylon scoparium* Pomel (syn. *Hammada scoparia* (Pomel) Iljin.) (Chenopodiaceae) with the aid of a functional assay with *Xenopus* oocytes transiently expressing $GABA_A$ receptors. Compound **62** represents the first report of a DHIC from the family Chenopodiaceae. On the basis of the recent report on a fungal isocoumarin, PF1223, as a ligand of the insect $GABA_A$ receptor with presumed inhibitory activity at the allosteric antagonist site, Li *et al*. in 2010 showed that **62** potentiated GABA-induced chloride current at the $GABA_A$ receptor with an EC_{50} of 140.2 (51.2μM) [47]. 3-Phenyl-substituted DHIC occur in plants of the families Asteraceae, Guttiferae, and Saxifragaceae.

FUNGI

Since 1933 a significant number of DHIC have been isolated and characterized from fungi species. The first DHIC isolated from fungi was mellein (**63**) (Fig. 18), a metabolic product of *Aspergillus melleus* (Trichocomaceae) [48].



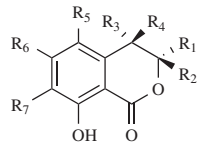


64

63 R_1=R_2=R_3=R_4=H
66 R_1=R_3= Cl R_2= OMe R_4=H
67 R_1=R_4= H R_2= OMe R_3=Cl
72 R_1= COOH R_2=R_3=R_4=H
76 R_1=Me R_2=R_4= H R_3= OMe
77 R_1=R_4= Me R_2=R_3=H
78 R_1= Me R_2=R_3=R_4=H
79 R_1=CH_2OH, R_2=R_3=R_4=H
89 R_1=R_2=R_3=H R_4=Me
90 R_1= CHO R_2=R_3=R_4=H
91 R_1=R_4= Me R_2=R_3=H
92 R_1=R_3= H R_2=OMe R_4=Me
93 R_1=Me R_2= R_4=H R_3=OH
99 R_1=R_2=R_4=H R_3=OH
102 R_1= OH R_2=R_3=R_4=H
103 R_1=Oisopentenyl R_2=R_3=R_4=H

FIGURE 18

Mellein is a 3-methyl-8-hydroxy-3,4-dihydroisocoumarin possessing a single chiral centre and it is present widely in fungi either in its *R* or *S* form, but mostly in its *R* forms. Most of the DHIC derivatives from fungi possess mellein skeleton. From the ethyl acetate extract of *A. terreus* culture subjected to repeated column chromatography and preparative thin layer chromatography on silica gel, the mellein derivatives **3**, **4**, 6-(4'-hydroxy-2'-methylphenoxy)-(3*R*)-mellein (**64**), and (3*R*,4*R*)-6,7-dimethoxy-4-hydroxymellein (**65**) were characterized (Figs. 2, 18, and 19) [49]. Compound **63** was also isolated from many *Pezicula* sp. (Dermateaceae), endophytic fungi that live inside Coniferous and deciduous trees and behave as plant hosts [50].

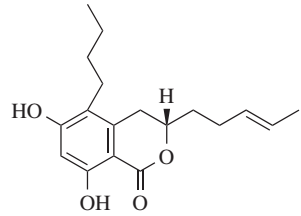


65 R_1=R_3=R_5=H R_2= Me R_4=OH R_6=R_7=OMe
69 R_1=R_3=R_5=R_6=R_7=H R_2= Me R_4=OH
70 R_1=R_4=R_5=R_7=H R_2=Me R_3=R_6=OH
71 R_1=Me R_2=R_4=R_5=R_7=H R_3=R_6=OH
100 R_1=Me R_2=R_4=R_5=R_6=H R_3=R_7=OH

FIGURE 19

Sondheimer *et al*. in 1957 isolated **3** from carrots which had developed a bitter taste during storage [51]. Condon *et al*. later associated the production of this fungitoxic substance in carrots with alterations in the normal metabolism of the carrot root tissue which they felt were possibly induced by the presence of fungi [52–54]. Successively, Aue *et al*. (1966) reported the isolation of the same compound, which is sometimes referred to the metabolism of the fungus *Sporormia bipartis* Cain. (Sporormiaceae) [55]. The study of the metabolic products of *S. affinis* Sacc., Bomm and Rouss conducted to the isolation together with **3** (Fig. 2), to the closely related halogenated compounds 5,7-dichloro-3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin (**66**) and 7-chloro-3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin (**67**) (Fig. 18) [56]. These findings and those of Aue *et al*. (1966) suggested that the occurrence of **3** in fungal infected carrots might be due to the fungus itself. The presence of **3** in two *Sporormia* species may also be noteworthy from a chemotaxonomic point of view [55].

During the examination of the metabolic products of a strain of *Fusarium* sp. (Nectriaceae), which was isolated from a natural source, a colourless, crystalline metabolite was isolated and identified as fusamarin (**68**) (Fig. 20) [57]. The isolation of the compound was obtained by ether extraction, followed by



68

FIGURE 20

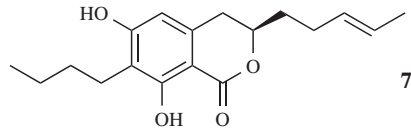
column chromatography and fractional recrystallization. Compound **68** has been shown to be (−)-(*R*)-(3)-*trans*-pentenyl-(3)-5-*n*-butyl-6,8-dihydroxy-3,4-dihydroisocoumarin on the basis of chemical and spectroscopic evidences.

Bioassay-guided fractionation of an ethyl acetate extract from the mycelium of the coprophilous fungus *Apiospora montagnei* Sacc. (JS140 = UAMH7489) (Amphisphaereaceae) has furnished the DHIC *cis*-(3*R*,4*R*)-4-hydroxymellein (**69**) (Fig. 19) [58,59], isolated also from *Aspergillus ochraceus*. The chemical similarity of this compound to the DHIC moiety of the ochratoxins implicates it as a possible biosynthetic precursor of the ochratoxins [58].

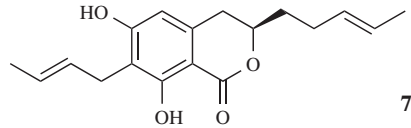
The isolation of the phytotoxic compounds from *Phomopsis helianthi* Munt-Cvetk. (Valsaceae) was performed by two consecutive semipreparative HPLC steps. Individual fractions were analyzed by analytical HPLC, and fractions with similar chromatographic profiles were combined and tested for phytotoxic activity on sunflower leaves by the puncture bioassay. The results of the bioassay led to the identification of two major metabolites. These compounds were identified by spectroscopic methods as *trans*-4,6-dihydroxymellein or *trans*-3-methyl-4,6,8-trihydroxy-3,4-dihydroisocoumarin (**70**) and *cis*-4,6-dihydroxymellein or *cis*-3-methyl-4,6,8-trihydroxy-3,4-dihydroisocoumarin (**71**) (Fig. 19). Both compounds showed phytotoxic activity towards sunflowers [60].

Cromatographic separation of compounds extracted from the culture of the strain *Tubercularia* sp. TF5 (Nectriaceae) led to isolation of 5-carboxymellein (**72**) (Fig. 18) successively isolated from different fungi species [61–63].

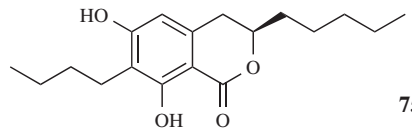
The crude extract of *Gentrichum* sp. (Endomycetaceae), an endophytic fungus isolated from *Crassacephalum crepidioides* S. Moore (Compositae), was found by Kongsaeree *et al*. (2003) to be active against *Plasmodium falciparum* (K1, multi-drug resistant strain) with an IC_{50} value of 0.63μg/mL and against *Candida albicans* with an IC_{50} of 19.2μg/mL. Bioassay-guided fractionation by Sephadex LH-20 and reversed-phase HPLC resulted in the isolation of three DHIC, whose structures were established as 7-butyl-6,8-dihydroxy-3(*R*)-pent-11-enylisochroman-1-one (**73**), 7-but-15-enyl-6,8-dihydroxy-3(*R*)-pent-11-enylisochroman-1-one (**74**), and 7-butyl-6,8-dihydroxy-3(*R*)-pentylisochroman-1-one (**75**) (Fig. 21) by spectroscopic data [64].



73



74

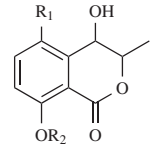


75

FIGURE 21

During a systematic screening by Kokubun *et al*. (2003) of fungi in the United Kingdom for antimicrobial and antiinsect activities, *Cytospora eucalypticola* van der Westhinger (Coelomycete) strain SS8 was found to produce antifungal and antibacterial metabolites and to release them into the growth medium. Fractionation of the culture filtrate yielded 3,5-dimethyl-8-hydroxy-7-methoxy-3,4-dihydroisocoumarin (**76**) and 3,5-dimethyl-8-methoxy-3,4-dihydroisocoumarin (**77**), together with 5-methylmellein (**78**), 5-hydroxymethylmellein (**79**), and 4-hydroxy-5-methylmellein (**80**) [65] (Figs. 18 and 22). The last three compounds have also been found in *C. eucalypticola*, the pathogen of *Eucalyptus* sp. [66], while compounds **77** and **78** were isolated by Ping *et al*. (2008) from endophytic fungus S26 of *Cephalotaxus hainanensis* Li (Cephalotaxaceae) [67].

During the course of a research project on biologically active compounds from fungi, the secondary metabolites produced by *Cephalosporium* sp. AL031 in culture were investigated by Bi *et al*. in 2004. The organism is a fungus that was isolated from *Sinarundinaria nitida* Keng F. ex T.P. Yi (Poaceae) grown in the Ailao Mountain, China. The phytochemical study of ethyl acetate extract led to the isolation of four glycosylated DHIC, elucidated as (2*E*,4*E*)-5-[(3*S*)-5-acetyl-8-(β-D-glucopyranosyloxy)-3,4-dihydro-6-hydroxy-1-oxo-1*H*-2-benzopyran-3-yl]penta-2,4-dienal (**81**), (2*E*,4*E*)-5-[(3*S*)-5-acetyl-8-(β-D-glucopyranosyloxy)-3,4-dihydro-6-methoxy-1-oxo-1*H*-2-benzopyran-3-yl] penta-2,4-dienal (**82**), (3*S*)-8-(β-D-glucopyranosyloxy)-3-[(1*E*,3*E*,5*E*)-hepta-1,3,5-trienyl]-3,4-dihydro-6-hydroxy-5-methyl-1*H*-2-benzopyran-1-one(**83**),and (3*S*)-8-[(6-*O*-acetyl-β-D-glucopyranosyl)oxy]-3-[(1*E*,3*E*,5*E*)-hepta-1,3,5-trienyl]-3,4-dihydro-6-methoxy-5-methyl-1*H*-2-benzopyran-1-one (**84**) by spectroscopic methods, including 2D-NMR techniques and chemical methods (Fig. 23) [68,69]. Successively, from the same fungus strain (2*E*)-3-[(3*S*)-5-acetyl-3,4-dihydro-6-methoxy-1-oxo-1*H*-2-benzopyran-3-yl]-2-propenoic acid



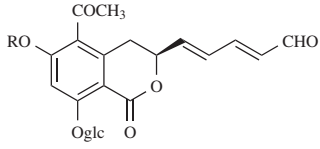
80 R_1= Me R_2=H
101 R_1=H R_2=Me

FIGURE 22

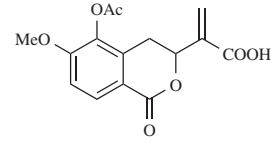
(**85**), (3*S*)-3,4-dihydro-3-heptantrienyl-8-hydroxyl-6-hydroxymethyl-5-methylisocoumarin (**86**), and (3*S*)-6-acetoxymethylene-3,4-dihydro-3-heptantrienyl-8-hydroxyl-5-methylisocoumarin (**87**), were isolated. Their structures were elucidated by interpretation of their spectroscopic data [70].

As a part of a continuing search for biologically active compounds from bioresources in Thailand [71–73], Berkaew *et al*. have investigated the constituents of the wood-decay fungus *Hypocrea* sp. BCC 14122 (Hypocreaceae), since an extract of this strain had shown activity against human small cell lung cancer cells (NCI-H187, IC_{50}=5.2μg/mL). The investigation on the EtOH extract led to the isolation of a DHIC (**88**) (Fig. 24) characterized by an alkyl side chain. The structure was elucidated primarily by NMR and mass spectroscopic analyses, but its absolute configuration was not determined [74].

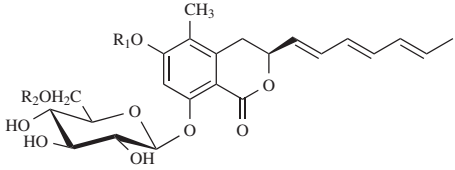
Four DHIC were isolated from the fungus *Nodulisporium* sp. by Kamisuki *et al*. (2007) and identified as 3-methyl-8-methoxy-3,4-dihydroisocoumarin (**89**), 5-formyl-8-hydroxy-3-methyl-3,4-dihydroisocoumarin (**90**), 3,5-dimethyl-8-methoxy-3,4-dihydroisocoumarin (**91**), 3-methyl-6,8-dimethoxy-3,4-dihydroisocoumarin or mullein (**92**) (Fig. 18), respectively, based on their physicochemical



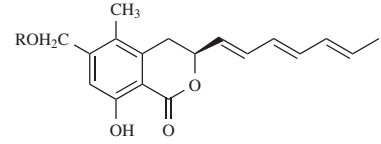
81 R = H
82 R = CH_3



85

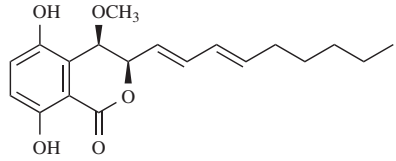


83 R_1 =R_2 =H
84 R_1=CH_3 R_2=Ac



86 R=H
87 R=Ac

FIGURE 23



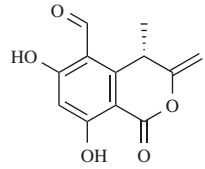
88

FIGURE 24

and spectroscopic data [75], while (3*R*)-7-hydroxy-5-methylmellein (**93**) (Fig. 18) was isolated from chemical investigation of the marine-derived fungi *Nodulisporium* sp. by Pontius *et al*. (2008) [76].

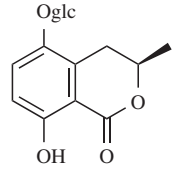
Krohn *et al*. in 2007 investigated the metabolites of an endophytic fungus, *Ascochyta* sp., isolated from the plant *Melilotus dentatus* Waldst e Kant (Fabaceae). The crude extract showed antifungal activity against *Microbotryum violaceum*, *Septoria tritici*, and *Phytophtora infestans*, as well as antibacterial activity against *Bacillus megaterium* and algicidal activity against *Chlorella fusca*. The ethyl acetate extract was subjected to column chromatography and the DHIC (4*S*)-(+)-ascochin (**94**) together with compound **69** (Figs. 19 and 25) were isolated [77,78]. Compound **94** had an unusual substitution pattern which was confirmed by X-ray diffraction.

The secondary metabolites produced by the *Aspergillus* species fungal strain CMM isolated from the sterilized twigs of *Cephalotaxus mannii* Hook. f. (Cephalotaxaceae) were investigated by Lu *et al*. in 2008 [79]. The chromatographic purification of the extracts from the fermentation products of the fungal strain led to the isolation of **69** and 5-*O*-α-D-glucopyranosyl-5-hydroxymellein (**95**) (Figs. 19 and 26) [80].



94

FIGURE 25



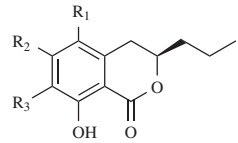
95

FIGURE 26

The genus *Phomopsis* (Valsaceae) is known to be a rich source of bioactive secondary metabolites of diverse structures such as xanthones [81,82], antifungal biaryl ethers (phomosines) [83,84], cycochalisines [85,86], convolvulanic acids [87], or mycotoxins such as phompopsin A [88]. Some of the compounds isolated from *Phomopsis* sp. exhibited significant *in vitro* antimalarial, antitubercular, and cytotoxic activities [82]. However, many of these bioactive compounds with antitumor and antibiotic activities have not yet found application due to toxicity problems [89]. In a series of preliminary screenings, the culture extract of the *Phomopsis* sp. displayed fungicidal, antibacterial, algicidal, and herbicidal activities [90]. The endophytic fungus *Phomopsis* sp. (internal strain no. 7233) was isolated from the leaves of *Laurus azorica* (Seub.) Franco (Lauraceae). In order to obtain the minor components, the fungus was cultivated, and three DHIC, named phomolactones A (**96**), B (**97**), and C (**98**) (Fig. 27), were obtained. Their structures, and in particular their stereochemistry, were elucidated by a thorough spectroscopic analysis in comparison to related hydroxycyclohexene epoxides and isocoumarins from the literature and to calculation results.

(3*R*)-7-Hydroxymellein (**99**) and (3*R,4R*)-4,7-dihydroxymellein (**100**) (Figs. 18 and 19), were isolated from *Botryosphaeria obtuse*, a pathogen of black dead diseases of grapevine. These DHIC exhibited a rare 7,8-dihydroxy substitution. The catechol moiety of **99** and **100** is probably formed after an additional oxidation at C-7, subsequently to the formation of the aromatic polyketide skeleton [91].

The mangrove endophytic fungus *Microsphaeropsis* sp. (Montagnulaceae) from the South China Sea was phytochemical investigated to obtain secondary metabolites. From this study eight DHIC derivatives were isolated from the ethyl acetate extract of the fermentation culture medium. Their structures were identified by comprehensive spectroscopic methods as 4-hydroxymellein (**69**), 5-carboxymellein (**72**), 5-methylmellein (**78**), 4-hydroxy-5-methylmellein (**80**), mullein (**92**), 7-hydroxymellein (**99**), 4-hydroxy-8-*O*-methylmellein (**101**), and 5-hydroxymellein (**102**) (Figs. 18, 19, and 22) [92].

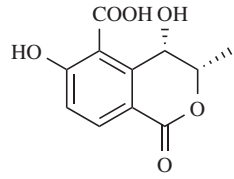


96 R_1=OH R_2=Cl R_3=H
97 R_1=OH R_2=R_3=H
98 R_1=R_2=H R_3=OH

FIGURE 27

From the ethyl acetate extract of the fermentation broth of the mangrove endophytic fungus *Cephalosporium* sp. a DHIC derivative containing an isoprenyl group, 5-(3'-methylbut-2'-enyloxy)-3,4-dihydro-8-hydroxy-3-methylisochromen-1-one (**103**) (Fig. 18), was isolated [93].

The DHIC (3*R*,4*R*)-3,4-dihydro-4,6-dihydroxy-3-methyl-1-oxo-1*H*-isochromene-5-carboxylic acid (**104**) (Fig. 28) was isolated from *Xylaria* sp. (Xylariaceae), a fungus associated with *Piper aduncum* L. (Piperaceae). Additionally, DHIC **99** and **100** (Figs. 18 and 19) were also isolated from *Penicillium* sp. (Trichocomaceae) associated to *Alibertia macrophylla* K. Schum (Rubiaceae) [94].

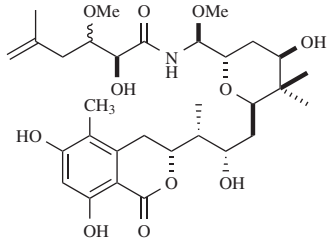


104

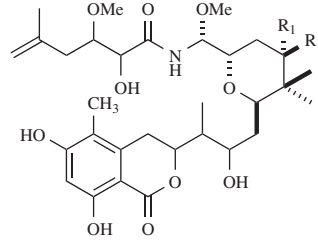
FIGURE 28

MARINE SOURCE

The DHIC moiety is present in the natural cytotoxin psymberin (**105**) (Fig. 29) reported from *Psammocinia* sp. (Dictyoceratida, Irciniidae) and in irciniastatin A (**106**) (Fig. 29) isolated from the marine sponge *Ircinia ramosa*. These compounds, independently described by Cichewicz *et al*. (2004) [95] and Pettit *et al*. (2004) [96] appeared to be diastereomeric. Irciniastatin B (**107**) was also isolated from *I. ramosa* [96]. The DHIC moiety seems to be essential for the differential cytotoxicity profile of psymberin [97].



105



106 R=OH R_1=H
107 R,R_1=O

FIGURE 29

PHARMACOLOGICAL AND BIOLOGICAL ACTIVITY

Antimicrobial Activity

Several DHICs have antimicrobial activity that in many cases is due to the presence of a phenolic group in their structure. Hydrangenol (**1**) and phyllodulcin (**2**) are among the first DHIC isolated in nature. Compound **2** possesses an extremely weak antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*, but a good activity against some fungi such as *Alternaria maritima*, *Cochliobolus miyabeanus*, *Fusarium splendens*, *Giberella zeae*, *Helminthosporum maydis*, and *Penicillum expansum* with MIC ranging between 50 and 100μg/mL [98]. The chemical modifications of phyllodulcin (**2**) structure such as acetylation, demethylation, or methylation of the hydroxyl group at the C-3' position resulted in a decrease of the antifungal activity. In addition, the structural modifications of hydrangenol (**1**), such as methylation at the C-4' position, causes decreasing of the antifungal effect. It was also found that optically active isomers showed stronger activity than the racemates [98].

The extracts of endophytic *Pezicula* species, isolated from different coniferous and deciduous trees, were tested for fungicidal, microbicidal, and algicidal activities [50]. Compound **63** was among the most active constituents isolated from these species, showing a strong fungicidal and herbicidal activity. The tested organisms were *B. megaterium* (Gram-positive bacteria), *E. coli* (Gram negative bacteria), *Ustilago violacea*, *Mycotypha microspora*, *Eurotium repens*, and *Fusarium oxysporum* fungi. Compound **63**, isolated in all investigated species of *Pezicula*, has also algicidal effect on the alga *C. fusca* [50].

Some free DHIC and their glycosides were tested against oral bacteria. DHIC from *H. macrophylla* Seringe var. *thunbergii* Makino were tested against *Bacteroides melaninogenicus* showing MIC of 10ppm for **1**, **14**, and **15**, 50ppm for **18**, and 100ppm for **2** and **24**, respectively, while the antibacterial activities against *Fusobacterium nucleatum* were 5ppm for **1**, 10ppm for **14** and **15**, 30ppm for **16**, and 100ppm for **2** and **24**, respectively [8]. So it seems that the free hydroxyl group at C-4' position is important to enhance the antibacterial activity.

A number of plants are used in the folk medicine for the treatment of skin diseases caused by pathogenic fungi. Compound **54**, isolated from the aerial parts *Xyris pteygoblephara* Steud. (Xyridaceae) growing in Brazil, was tested at the concentration of 100μg/disc against clinical isolates of dermatophytes *Epidermophyton floccosum* showing inhibition zone of 4.5 ± 0.8mm, *Trichophyton mentagrophytes* showing inhibition zone of 4.8 ± 0.4mm, and *Trichophyton rubrum* revealing an inhibition zone of 10.2 ± 0.8mm, confirming the ethnopharmacological use of this plant for the treatment of skin fungal diseases [39].

Compounds **58**–**60** isolated from *C. biafrae* S. Moore (Asteraceae) were tested (1mg/mL) against a range of microorganisms such as *E. coli, Bacillus subtilis, S. aureus, Pseudomonas picketti, Trichophyton longifusus, Aspergillus*

flavus, Microsporum canis, Fusarium solani, C. albicans, and *Candida glabrata* by the agar-well diffusion method. All compounds were active against *B. subtilis* and *E. coli*. Compounds **58**–**60** (200μg/mL) were also screened *in vitro* for the antifungal activity against six fungi species using the tube diffusion test. The linear growth of the fungus was obtained by measuring the diameter of the fungal colony after seven days. Compounds **58** and **59** exhibited significant activity (>75%) against *C. albicans*, *F. solani*, and *T. longifusus*, while **60** showed significant activity (80%) against *F. solani* [44].

Compounds **73**–**75** isolated from *Geotrichum* sp. were tested against *C. albicans* and *Mycobacterium tuberculosis*. The IC_{50} was 19μg/mL for compound **73** and 33μg/mL for **75**, respectively. Results were compared with standard drugs such as amphotericin B (IC_{50} = 0.01μg/mL).

Growth inhibition activity against *M. tuberculosis* H37Ra strain was determined using the Microplate Alamar Blue Assay (MABA). The MIC values of the standard drugs isoniazid and kanamycin sulphate were 0.050 and 2.5μg/mL, respectively, while the active DHIC were **73** and **74** with MIC of 25 and 50μg/mL, respectively [63]. The *in vitro* antimalarial activity was also evaluated against the parasite *P. falciparum* (K1, multi-drug resistant strain). Quantitative assessment of antimalarial activity was determined by means of the microculture radioisotope. An IC_{50} value of 4.7μg/mL and 2.6μg/mL was observed for compounds **73** and **75**, respectively [64].

Compounds **69** and **94** isolated from *Ascochyta* sp. were tested against the Gram-positive bacterium *B. megaterium*, the fungus *M. violaceum*, and the algae *C. fusca*. Compound **68** was particularly active against bacteria [77].

Finally, compounds **99** and **104** were evaluated for their antifungal activity against *Cladosporium sphaerospermum* and *C. cladosporioides*, using direct bioautography assay. Compound **99** showed a potent effect against the yeasts, exhibiting a detection limit of 5.0 and 10.0μg against *C. cladosporioides* and *C. sphaerospermum*, respectively. Compound **104** exhibited moderate fungitoxicity towards *C. cladosporioides* and *C. sphaerospermum*, showing a detection limit of 10.0 and 25.0μg, respectively. Altogether, these data indicate a clear positive correlation between the antifungal effect demonstrated by the DHIC and their catechol moiety. Compound **99** also showed a limit of detection of 10μg in a TLC-based AChE inhibition assay which was considered as weakly potent. Compound **104** displayed moderate AChE inhibitory activity, exhibiting a detection limit of 3.0μg [94].

Chemopreventive Activity

Recent researches have suggested that the presence of plant secondary metabolites in diet could reduce the risk of cancer. Particularly, phenolic compounds are well known for their antioxidant properties. Some isolated DHICs were tested in different antioxidant assays. Compound **51**, isolated from the whole plant of *P. hongkongensis* Hemsl., showed an IC_{50} of 33.3μM in DPPH radical

scavenging activity test, 56.6μM in hydroxyl radical scavenging activity test, and 78.μM in reductive activity to Fe^{3+} [37].

DHIC **54** isolated from the aerial parts of *X. pterygoblephara* Steud. (Xyridaceae) has shown aromatase inhibitory activity, yielding an IC_{50} value of 1.6 ± 0.1μM. Aromatase, a key cytochrome P450 enzyme, is responsible for the biosynthesis of estrogens which play an important role in the promotion of certain cancers, such as breast and endometrial cancer. Therefore, **54** could be employed as a prototype for future development which may result in a new chemopreventive agent [99].

Compound **1** showed an inhibitory effect on azoxymethane-induced colonic aberrant crypt foci and colonic mucosal ornithine decarboxilase activity in rats, indicating that it may have an antitumoral potential. Therefore **1** was suggested to be a candidate for chemopreventive agents in colon tumorigenesis [100].

Allergic and Antiallergic Activities

Many cases of contact dermatitis are reported for *Hydrangea* species. These plants could be considered an occupational dermatosis among nursery workers, presenting as an eczema involving the hands [101]. Patch tests with the stems as well as the leaves of their extracts gave strong positive reactions in all patients, and **1**, the allergen of *Hydrangea*, when tested, dissolved in acetone at the concentration of 0.01, 0.1, and 1%, gave positive results in the allergic test [102].

Surprisingly, another study suggested that **1**, besides other DHIC, is a weak inhibitor of histamine release in rat peritoneal mast cells induced by antigen–antibody reaction [103]. A further study showed that **1** and **2** also showed significant inhibition for the antigen-induced degranulations. The antiallergic effects of these active compounds seem to be due to the inhibition on antigen-induced release of β-hexosaminidase [104].

DHIC in Food

Phenolic derivatives are among the most important food bioactive metabolites because of their antioxidant activity. In addition some phenolics have sweetener properties.

Arakawa in 1960 studied the absolute configuration of compound **2**, showing that the *R*-isomer was from 400 to 800 times sweeter than sucrose [105]. Later on, it was showed that the β-D-glucoside of phyllodulcin (**23**) was not sweet and for this reason in the fermentation process it is first necessary to hydrolyze phyllodulcin from its glucosidic form to obtain the intense sweet taste of the aglycon [7]. Although **2** has been rated as 400 times as sweeter than a 3% (w/v) solution of sucrose, its wider use as a low caloric sweetener is restricted by a late onset of sweetness, insufficient solubility in water, and a lingering aftertaste [106].

DHIC were found in the aroma of some minor tropical fruits; compound **63** is one volatile DHIC present in the aroma of *Theobroma grandifora* (Willd. ex

Spreng.) K. Schum (Sterculariaceae) which is known in South America countries as "cupuaçu" and mainly consumed fresh, as juice or as dessert [107].

Some vegetables such as carrots under certain circumstances produce DHIC with bitter taste. The presence of 6-methoxymellein (**3**), 6-hydroxymellein (**6**), and 3-methyl-6-methoxy-3,4-dihydroisocoumarin-8-*O*-β-D-glucopyranoside (**56**) was reported in carrot tissues. These compounds were predominantly accumulated in the periderm tissue of carrot roots. The biosynthesis of **3** is stimulated by exposure to ethylene and to UV radiation, by microbial infection, wounding, and storage at elevated temperatures [41,108]. These compounds with bitter taste have a defence role in the plants as a response to infection or injury. Moreover, carrot roots infected by pathogens *Mycrocentrospora acerina* and *Botrytis cinerea* accumulated excess of phenolic antifungal **3** [109].

DHIC FROM ANIMAL ORIGIN

DHIC are not found only in the plant kingdom but also in some phylum of animalia, including sponges and insects.

Some insects such as the carpenter ants *Camponotus herculeanus* and *Camponotus ligniperda* produced mellein (**63**) from their mandibular glands especially during the swarming process used for attracting the females. The role of **63** is the opposite in other insects, such as the red clover thrip *Huplothrips leucanthemi* which use it as defence arsenal with deterrent efficacy for fire ant workers *Solenopsis invicta*. This activity was examined by adding 0.5 or 1μg of mellein in 2μL of ethanol to a droplet of honey [110].

Compound **63** was detected also in *Camponotus ramulorum* [111].

REFERENCES

[1] Y. Asahina, K. Miyake, Yakugaku Zasshi 408 (1916) 121.
[2] Y. Asahina, S. Ueno, Yakugaku Zasshi 408 (1916) 146.
[3] T.R. Govindachari, S.J. Patankar, N. Viswanathan, Phytochemistry 10 (1971) 1603–1606.
[4] K. Inoue, H. Inouye, C.-C. Chen, Phytochemistry 20 (1981) 2271–2276.
[5] K.M. Biswas, H. Mallik, Phytochemistry 25 (1986) 1727–1730.
[6] Y. Asakawa, Y. Tada, T. Hashimoto, Phytochemistry 37 (1994) 233–235.
[7] R. Zehneter, H.; Gerlach, Tetrahedron Asym. 6 (1995) 2779–2786.
[8] M. Yoshikawa, E. Uchida, N. Chatani, H. Kobayashi, Y. Naitoh, Y. Okuno, H. Matsuda, J. Yamahara, N. Murakami, Chem. Pharm. Bull. 40 (1992) 3352–3354.
[9] M. Yoshikawa, T. Murakami, T. Ueda, H. Shimoda, J. Yamahara, H. Matsuda, Heterocycles 50 (1999) 411–418.
[10] M. Yoshikawa, T. Ueda, H. Shimoda, T. Murakami, J. Yamahara, H. Matsuda, Chem. Pharm. Bull. 47 (1999) 383–387.
[11] T. Hashimoto, M. Tori, Y. Asakawa, Phytochemistry 26 (1987) 3323–3330.
[12] G. Speranza, P. Manitto, P. Cassara, D. Monti, Phytochemistry 33 (1993) 175–178.
[13] N.C. Veitch, M.J. Simmonds, W.M. Blaney, T. Reynolds, Phytochemistry 35 (1994) 1163–1166.
[14] H.M. Wang, W. Shi, Y.K. Xu, Y. Liu, M.J. Lü, J.Q. Pan, Magn. Reson. Chem. 41 (2003) 718–720.

[15] Y. Yang, H.M. Wang, L. Guo, Y. Chen, Biomed. Chromatogr. 18 (2004) 112–116.
[16] A. San Feliciano, A.F. Barreo, M. Medarde, J.M.M. Del Corral, M.V. Calle, Phytochemistry 22 (1983) 2031–2033.
[17] A. San Feliciano, J.M.M. Del Corral, L.M. Canedo, M. Medarde, Phytochemistry 29 (1990) 945–948.
[18] A.F. Barrero, J.F. Sanchez, I. Rodriguez, Phytochemistry 29 (1990) 1967–1969.
[19] N.H. Rama, A. Saeed, C.W. Bird, Liebigs Ann. Chem. (1993) 1331–1333.
[20] M.T. Hussain, A. Saeed, N.H. Rama, A.R. Raza, C.W. Bird, J. Chem. Soc. Pak. 23 (2001) 38–41.
[21] M. Yamato, K. Hashigaki, Chem. Pharm. Bull. 24 (1976) 200–203.
[22] E.E.-J. Park, H. Oh, T.-H. Kang, D.-H. Sohn, Y.C. Kim, Arch. Pharm. Res. 27 (2004) 944–946.
[23] H. Kato, W. Li, M. Koike, Y. Wang, K. Koike, Phytochemistry 71 (2010) 1925–1929.
[24] T.-S. Wu, C.-C. Hwang, P.-C. Kuo, T.-H. Kuo, A.G. Damu, C.-R. Su, Chem. Pharm. Bull. 52 (2004) 1227–1230.
[25] N.X. Cuong, N.X. Nhiem, N.P. Thao, N.H. Nam, N.T. Dat, H.L.T. Anh, L.M. Huong, P.V. Kiem, C.V. Minh, J.-H. Won, W.-Y. Chung, Y.H. Kim, Bioorg. Med. Chem. Lett. 20 (2010) 4782–4784.
[26] K. Sakai, Y. Naoi, T. Nakano, H. Ito, S. Higuchi, S. Wagatsuma, Y. Takahashi, T. Matsui, A. Nishi, S. Sano, Japan Kokai Tokkyo Koho JP 74110668(741022); CA: 83(1)9789k.
[27] C. Ito, Y. Mishina, M. Litaudon, J.-P. Cosson, H. Furukawa, Phytochemistry 53 (2000) 1043–1046.
[28] D. Lu, C. Wang, J. Liu, S. Chen, P. Li, Zhongcaoyao 38 (2007) 1785–1787.
[29] S. Paraschos, P. Magiatis, E. Kalpoutzakis, C. Harvala, A.-L. Skaltsounis, J. Nat. Prod. 64 (2001) 1585–1587.
[30] A. Sari, C. Zidorn, E.P. Ellmerer, F. Özgökçe, K.-H. Ongania, H. Stuppner, Helv. Chim. Acta 90 (2007) 311–317.
[31] G. Saltan çitoğlu, Ö Bahadir, S. Dall'Acqua, Turk. J. Pharm. Sci. 7 (2010) 205–212.
[32] A. Bader, N. De Tommasi, R. Cotugno, A. Braca, J. Nat. Prod. 74 (2011) 1421–1426.
[33] C. Zidorn, U. Lohwasser, S. Pschorr, D. Salvenmoser, K.-H. Ongania, E.P. Ellmerer, A. Börner, H. Stuppner, Phytochemistry 66 (2005) 1691–1697.
[34] C. Zidorn, B.O. Petersen, V. Sareedenchai, E.P. Ellmerer, J.Ø. Duus, Tetrahedron Lett. 51 (2010) 1390–1393.
[35] T. Mahmood, E. Ahmed, A. Malik, Magn. Reson. Chem. 43 (2005) 670–672.
[36] Y. Li, F. Luo, S. Peng, J. Liang, L. Ding, Nat. Prod. Res. 20 (2006) 860–865.
[37] J.-F. Wu, S.-B. Chen, J.-C. Gao, H.-L. Song, L.-J. Wu, S.L. Chen, P.-F. Tu, Nat. Prod. Res. 21 (2007) 580–584.
[38] A.A. Magid, L. Voutquenne-Nazabadioko, G. Moroy, C. Moretti, C. Lavaud, Phytochemistry 68 (2007) 2439–2443.
[39] K.G. Guimarães, J.D. de Souza Filho, T.R. dos Mares-Guia, F.C. Braga, Phytochemistry 69 (2008) 439–444.
[40] G. Gao, S. Qi, S. Zhang, H. Yin, Z. Xiao, M. Li, Q. Li, Pharmazie 63 (2008) 542–544.
[41] J.-X. Liu, D.-L. Di, Y.-P. Shi, J. Chin. Chem. Soc. 55 (2008) 863–870.
[42] P. Valentao, P.B. Andrade, A.M.S. Silva, M.M. Moreira, R.M. Seabra, Nat. Prod. Res. 17 (2003) 361–364.
[43] O. Jovanović, N. Radulović, G. Stojanović, R. Palić, J. Essent. Oil Res. 21 (2009) 317–322.
[44] T.K. Tabopda, G.W. Fotso, J. Ngoupayo, A.C. Mitaine-Offer, B.T. Ngadjui, M.A. Lacaille-Dubois, Planta Med. 75 (2009) 1258–1261.
[45] P.T. Nedialkov, D. Zheleva-Dimitrova, U. Girreser, G.M. Kitanov, Nat. Prod. Res. 21 (2007) 1056–1060.

[46] A. Saeed, J. Asian Nat. Prod. Res. 12 (2010) 88–93.
[47] Y. Li, I. Plitzko, J. Zaugg, S. Hering, M. Hamburger, J. Nat. Prod. 73 (2010) 768–770.
[48] E. Yukawa Nishikawa, Nippon Nogei Kagaku Kaishi 9 (1933) 1059–1063.
[49] M.I. Choudhary, S.G. Musharra, T. Mukhmoor, F. Shaheen, S. Ali, Atta-ur-Rahman, Zeit. Naturforsch. B Chem. Sci. 59 (2004) 324–328.
[50] B., Schultz, J. Sucker, H.J. Aust, K. Krohn, K. Ludewig, P.G. Jones, D Doring, Mycol. Res. 40 (1995) 3121–3123.
[51] E. Sondheimer, J. Amer. Chem. Soc. 79 (1957) 5036.
[52] P. Condon, J. Kue, Phytopathology 50 (1960) 267.
[53] P. Condon, J. Kue, Phytopathology 52 (1962) 182.
[54] P. Condon, J. Kue, M.H. Draudt, Phytopathology 55 (1963) 1244.
[55] R. Aue, R. Mauli, R.P. Sigg, Experientia 22 (1966) 575.
[56] W.J. McGahren, L.A. Mitscher, J. Org. Chem. 33 (1968) 1577–1580.
[57] Y. Suzuku, Agr. Biol. Chem. 34 (1970) 760–766.
[58] R.J. Cole, J.H. Moore, N.D. Davis, J.W. Kirksey, U.L. Diener, J. Agric. Food Chem. 19 (1971) 909–911.
[59] A.A. Alfatafta, J.B. Gloer, J. Nat. Prod. 57 (1994) 1696–1702.
[60] G. Avantaggiato, M. Solfrizzo, L. Tosi, A. Zazzerini, F.P. Fanizzi, A. Visconti, Nat. Toxins 7 (1999) 119–127.
[61] A.D.A. Marden, B.F. Raimundo, R.G. Otto, Phytochemistry 17 (1978) 511–516.
[62] F.B. Sean, M.B. Shana, C. Jon, J. Am. Chem. Soc. 123 (2001) 9900–9901.
[63] Y. Li, C. Lu, Z. Hu, Y. Huang, Y. Shen, Nat. Prod. Res. 23 (2009) 70–76.
[64] P. Kongsaeree, S. Prabpai, N. Sriubolmas, C. Vongvein, S. Wiyakrutta, J. Nat. Prod. 66 (2003) 709–711.
[65] T. Kokubun, N.C. Veitch, P.D. Bridge, M.S.J. Simmonds, Phytochemistry 62 (2003) 779–782.
[66] T. Okuno, S. Oikawa, T. Goto, K. Sawai, H. Shirahama, T. Matsumoto, Agric. Biol. Chem. 50 (1986) 997–1001.
[67] C. Ping, J. Wu, H. Dai, X. Xie, W. Mei, Zhongguo Yaowu Huaxue Zazhi 18 (2008) 279–283.
[68] Y.-M. Bi, Y.-T. Chen, J.-L. Xie, H.-Q. Wang, Chin. J. Pharm. 32 (2001) 488.
[69] Y.-M. Bi, X.-B. Bi, Q.-R. Zhao, Y.-T. Chen, J.-L. Xie, Hel. Chim. Acta 87 (2004) 2890–2895.
[70] Y.-M. Bi, X.-B. Bi, Y.-T. Chen, J.-L. Xie, Nat. Prod. Res. 19 (2005) 425–428.
[71] N. Soonthornchareonnon, M. Sakayarojkul, M. Isaka, V. Mahakittikun, W. Chuakul, P. Wongsinkongman, Chem. Pharm. Bull. 53 (2005) 241–243.
[72] M. Isaka, W. Prathumpai, P. Wongsa, M. Tanticharoen, Org. Lett. 8 (2006) 2815–2817.
[73] M. Isaka, P. Berkaew, K. Intereya, S. Komwijit, T. Sathitkunanon, Tetrahedron 63 (2007) 6855–6860.
[74] P. Berkaew, N. Soonthornchareonnon, K. Salasawadee, R. Chanthaket, M. Isaka, J. Nat. Prod. 71 (2008) 902–904.
[75] S. Kamisuki, C. Ishimaru, K. Onoda, I. Kuriyama, N. Ida, F. Sugawara, H. Yoshida, Y. Mizushina, Bioorg. Med. Chem. 15 (2007) 3109–3114.
[76] A. Pontius, I. Mohamed, A. Krick, S. Kehraus, G.M. Konig, J. Nat. Prod. 71 (2008) 272–274.
[77] K. Krohn, R. Bahramsari, U. Florke, K. Ludewig, C. Kliche-Spory, A. Michel, H.J. Aust, S. Draeger, B. Schulz, S. Antus, Phytochemistry 45 (1997) 313–320.
[78] U. Holler, G.M. Konig, A.D. Wright, J. Nat. Prod. 62 (1999) 114–118.
[79] C. Lu, X. Lin, Y. Shen, Chem. Nat. Compd. 44 (2008) 569–571.
[80] P. Venkatasubbalah, W.S. Chilton, J. Nat. Prod. 53 (1990) 1628.
[81] V. Rukachaisirikul, U. Sommart, S. Phongpaichit, J. Sakayaroj, K. Kirtikara, Phytochemistry 69 (2008) 783–787.

[82] M. Isaka, A. Jaturapat, K. Rukseree, K. Danwisetkanjana, M. Tantichareon, Y. Thebtaranonth, J. Nat. Prod. 64 (2001) 1015–1018.
[83] K. Krohn, A. Michel, E. Roemer, U. Flörke, H.J. Aust, S. Draeger, B. Schulz, V. Wray, Nat. Prod. Lett. 9 (1995) 309–314.
[84] J. Dai, K. Krohn, U. Flörke, D. Gehle, H.J. Aust, S. Draeger, B. Schulz, K. Rheinheimer, Eur. J. Org. Chem. 23 (2005) 5100–5105.
[85] W.S. Horn, M.S.J. Simmonds, R.E. Schwartz, W.M. Blaney, Tetrahedron 51 (1995) 3969–3978.
[86] Y. Izawa, T. Shimuzu, K. Koyama, S. Natori, Tetrahedron 45 (1989) 2323–2335.
[87] Y.S. Tsantrizos, K.K. Ogilvie, A.K. Watson, Can. J. Chem. 70 (1992) 2276–2284.
[88] C.C.J. Culvenor, J.A. Edgar, W.F.O. Marasa, M.F. Mackay, C.P. Gorst-Allman, P.S. Steyn, R. Vleggaar, P.L. Wessela, Tetrahedron 45 (1989) 2351–2372.
[89] G.A. Strobel, B. Daisy, U. Castillo, J. Harper, J. Nat. Prod. 67 (2004) 257–268.
[90] H. Hussain, N. Akhtar, S. Draeger, B. Schulz, G. Pescitelli, P. Salvadori, S. Antus, T. Kurtàn, K. Krohn, Eur. J. Org. Chem. (2009) 749–756.
[91] J.D. Djoukeng, S. Polli, P. Larignon, E. Abou-Mansour, Eur. J. Plant. Pathol. 124 (2009) 303–308.
[92] M. Wie, G. Hu, C. Zheng, C. Shao, C. Wang, S. Zhou, Z. She, Y. Lin, Zhongshan Daxue Xuebao, Ziran Kexueban 49 (2010) 68–71.
[93] M. Wie, X. Zhang, S. Li, C. Shao, C. Wang, Z. She, Y. Lin, Chem. Nat. Prod. 46 (2010) 340–342.
[94] C.M. Oliveira, L.O. Regasini, G.H. Silva, L.H. Pfenning, M.C.M. Young, R.G.S. Berlinck, V.S. Bolzani, A.R. Araujo, Phytochem. Lett. 4 (2011) 93–96.
[95] R.H. Cichewicz, F.A. Valeriote, P. Crews, Org. Lett. 6 (2004) 1951–1954.
[96] G.R. Pettit, J. Xu, J. Chapuis, R.K. Pettit, L.P. Tackett, D.L. Doubek, J.N.A. Hooper, J.M. Schmidt, J. Nat. Prod. 47 (2004) 1149–1152.
[97] X. Jiang, N. Williams, J.K. De Brabander, Org. Lett. 9 (2007) 227–230.
[98] K. Nozawa, M. Yamada, Y. Tsuda, K. Kawai, S. Nakajima, Chem. Pharm. Bull. 29 (1981) 2689–2791.
[99] D.C. Endringer, K.G. Guimarães, T.P. Kondratyuk, J.M. Pezzuto, F.C. Braga, J. Nat. Prod. 71 (2008) 1082–1084.
[100] T. Kawamori, T. Tanaka, A. Hara, J. Yamahara, H. Mori, Cancer Res. 55 (1995) 1277–1282.
[101] B.M. Hausen, Contact Dermatitis 24 (1991) 233–235.
[102] M. Avenel-Audran, B.M. Hausen, J. Le Sellin, G. Ledieu, J.L. Verret, Contact Dermatitis 43 (2000) 189–191.
[103] H. Matsuda, H. Shimoda, M. Yoshikawa, Bioorg. Med. Chem. 7 (1999) 1445–1450.
[104] O. Wang, H. Matsuda, K. Matsuhira, S. Nakamura, D. Yuan, M. Yoshikawa, Biol. Pharm. Bull. 30 (2007) 338–392.
[105] H. Arakawa, Bull. Chem. Soc. Jpn. 33 (1960) 200–202.
[106] M. Behrens, W. Meyerhof, C. Hellfritsch, T. Hofmann, Angew. Chem. Int. 50 (2011) 2220–2242.
[107] M.R.B. Franco, N.S. Janzantti, J. Flav. Frag. 20 (2005) 358–371.
[108] M. Naczk, F. Shahidi, J. Pharm. Biomed. Anal. 41 (2006) 1532–1542.
[109] I. Babic, M.J. Amiot, C. Nguyen-The, S. Aubert, J. Food Sci. 58 (1993) 351–356.
[110] S.B. Murray, R. Foottit, H.M. Fales, Comp. Biochem. Physiol. 102 (1992) 209–2011.
[111] J.A. Torres, R.R. Snelling, M.S. Blum, R.C. Flournoy, T.H. Jones, R.M. Duffield, Biochem. Syst. Ecol. 29 (2001) 673–680.

Chapter 8

The Modern Analytical Determination of Botanicals and Similar Novel Natural Products by the HPTLC Fingerprint Approach

Marcello Nicoletti[a], Valentina Petitto[a], Francesca R. Gallo[b], Giuseppina Multari[b], Elena Federici[b] and Giovanna Palazzino[b]

[a]*Department of Environmental Biology, University Sapienza of Rome, P.le A. Moro 5I-00185, Rome, Italy*

[b]*Department of Therapeutic Research and Medicines Evaluation, Istituto Superiore di Sanità, V.le Regina Elena 299I-00161, Rome, Italy*

INTRODUCTION

A Long and Winding Road

Something more than a century ago, like Alice in a wonderland, pathfinders of the natural world received a little key to explore the magical garden of plant molecular structures and named it chromatography. Opened the small door, they found a narrow passage and an enormous work waiting, with unforeseen blossoms to discover and intriguing pathways to detect. Fascinated they tried to scan every little corner of the garden, to draw the entire map and be costumers; always they were indifferent to hard works, difficulties and dangers. Analytical organic chemistry gained from Phytochemistry two important legacies: an immense catalogue of astonishing structures of natural compounds isolated from a large quantity of plant species and an armoury of powerful implements and devices. They were the result of the classic sequence extraction/purification/structural determination and the fruits of an intelligent persistent effort. They were useful to make up the necessary ground for any further progress in natural products chemistry and commercial use of natural substances. Clearly, the main aim of the phytochemical study, as well as the finalization of the analytical determination, was the pursuit of active principles with pharmacological activity to be used by the pharmaceutical industry or directly or as raw material for hemisynthesis or as leader compounds for syntheses of

Bioactive Natural Products (Part Q), Vol. 37. http://dx.doi.org/10.1016/B978-0-444-59514-0.00008-0

new drugs. Consequently, a natural product was relevant for novelty in structure, because of promising activity in pharmacological tests and in the consideration of the possible use as a medicament.

Rari nantes in gurgite vasto

Virgil, Aeneid, I, 118

Phytochemical students were pearl divers, lost in the huge billows of the secondary metabolism. A challenge so hard as fascinating. Later on, a devilish alliance of bureaucracy and claims killed any hope of novelty. Nowadays, a new active product means an unsustainable quantity of money to satisfy patents, certifications and endless clinical tests.

However, the request for natural substances has been increasing and demands changing progressively, together with goals and points of view. Avowal of the importance of the phytocomplex and the holistic view gave rise first to a new therapeutic approach, and hence to come out of new forms of natural products utilizations. Debate is going on about activity and real need of food supplements in diet, including a plethora of negative opinions that did not stop the boom of these products. Waiting for validations from the System Biology, the new products immediately filled neglected spaces and those arose, facing a great agreement in the market. Starting as products dedicated to particular uses, e.g., to satisfy dietetic needs or to improve the sport performances, food supplements, and in particular botanicals, overflew entering in the ordinary life of everybody. Following this trend, next step will be the multifunctional food, considered as "any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains." Decision Resources, Inc. [1] estimates the market value of functional foods at $28.9 billion. The potential of functional foods is, perhaps, more significant to mitigate risks of diseases, to promote health and to reduce health care costs.

Borderlines in the Food Market

The first signal was the insistent appearance on the market of several new products characterised by elements of novelty, as well as of confusion, including a profusion of imaginative denominations, i.e., nutraceuticals, food supplements, botanicals, dietary supplements, multifunctional foods, herbal drug preparations and others [1]. Rapidly, the new products entered *de facto* in the mainstream of health commodities. These are modern forms of natural substances utilization, inheriting the natural origin from the tradition cares like herbal medicines and herbalist products and offering solutions for the treatment of increasing minor pathologies or needs for health maintenance. Within the EU market for the food supplements, vitamins and minerals account for the largest share (about 50%–55%: €4.1 – €4.73 billion), with the balance (€3.87 – €4.1 billion) accounting for "other substances", mainly due to extracts of plants and their constituents [2].

In 2009 in Italy, one third of the population used food supplements and related products [3]; the amount of money involved was about €1.5 billion

(12.5% more than 2008) and the products amounted for €119 millions (an increment of 7.5%).

Searching for Quality

The market of "other substances", after the first period of enthusiastic explosive emerging, is entering into the maturation period, with three important aspects to face: (a) security in composition, production and selling, avoiding easy conversion and new approaches and meanwhile favouring competence and expertise; (b) definition of influence on metabolic aspects, including the scientific validation; (c) regulatory aspects, e.g., the claims definition and relative influences. The last aspect seems to be the most crucial and fundamental to the future of all the sectors.

Botanicals urgently need adequate analytical controls [4], since their uptrend could continue and be insured only by quality assessments. To handle complex analytical tasks with herbal drugs and botanicals is completely different from identifying single substances: (a) the exact chemical composition of botanical products is largely unknown and may widely vary; (b) the quantitative presence of known active or marker compounds is not the unique qualitative criterion, since the presence of other constituents must also be a part of the analysis of the sample; (c) minimal variations in featuring conditions can heavily influence the analytical result and their complicate interpretation and comparison.

Phytochemical studies began from chromatography that opened the doors of the micromolecular world. From the beginning, started with the first application by Michael Tswett [5,6], the future of the chromatography was marked: it was suited for separation and identification of plant constituents. The central position must be conceived to the planar chromatography, in particular the Thin Layer Chromatography (TLC) [7]. Nowadays, TLC remains the most immediate, the simplest, the most useful analytical friend in the laboratory of organic chemistry to check the presence and the identity of known marker compounds, to follow the improvement of a synthesis, to test separation trend in column fractions and others. However, the current challenge of complex mixtures, as those present in the botanicals, needs more effective proper analytical tools. The limits of TLC to face and solve the complex mixtures problems were the basis of its positive evolution. TLC planar chromatography is: (a) an open system, depending on environmental factors (temperature, light, fumes, humidity), that can influence the resulting data; (b) mainly based on critical manual steps, influencing the results also using the best rigour and despite all the extra cautions of the same operator; (c) interpretation based on visualization can be subjected to personal judgement. Therefore, the TLC methods cannot be fully controlled and analyses are not totally reproducible. In other words, data are scientifically not totally reliable and confident. TLC still remains a craftsman's performance.

The Chromatography of the Twenty-First Century

High Performance Thin Layer Chromatography (HPTLC) is the most recent evolution of planar chromatography, whose mission is to change the weakness of TLC into strength [8,9].

HPTLC raised from a need for major separation capacity, obtained by the use of precoated plates with smaller particles (2μm vs. 15μm), i.e., a more active surface, in order to obtain the efficacy needed for plant mixtures. In the modern HPTLC, the plate is the central tool of a complex automatic instrumentation system developed to control analysis conditions, to optimize reproducible results and to allow a complete comparison between different laboratories. In this regard, HPTLC is much more complicated than TLC, but complex problems need complex solutions. Being a multistep process, HPTLC performance requires a separated device for each step of the sequence: sample application, chromatogram development, derivatization, visualization and documentation. Furthermore, quantitative data for each spot can be obtained by densitometric measurements. The full power of HPTLC comes from the proper, compatible and complementary use of each device in an integrated system. The operator is the director of a tentative to reproduce Nature's symphony tricking with sophisticated machines.

HPTLC Methodology

Birth of modern planar chromatography can be placed in 1958 at the Achema trade fair in Frankfurt, with the presentation of the first TLC plates. Parents were Egon Stahl and the firm Merck. HPTLC plates were available about 20 years later by the study of R.F. Kaiser. Merck, (Darmstadt, Germany) still remains a leader in production of TLC plates with high capacity of separation, including those utilised in the applications here reported (HPTLC Silica gel 60 F_{254} 20×10, cat no. 1.05642.0001).

Instrumentation improvements played a major role in turning conventional TLC into HPTLC with high quality results. The analyses here reported were performed through the following steps and related devices by Camag (Muttenz, Switzerland): (a) each sample is deposited by a syringe of an automated device (Linomat 5) that allows to select and control form, concentration and type of the starting spots, as well as the proper distances, insuring the same best starting conditions; (b) plates are run in special development saturated chambers. Silica gel is hygroscopic and always in equilibrium with the humidity of the laboratory atmosphere, that can affect the activity of silica. Therefore, using automated developing chambers (ADC2) to control conditions such as humidity, chamber saturation with mobile-phase vapour and extent of mobile-phase migration can make a big difference in reproducibility of results. They may also include sensors to detect the advance of the solvent front, so a plate can be automatically withdrawn and dried at a specific point; (c) the plate can be

visualised in different conditions (TLC Visualizer) and derivatised in controlled conditions (Chromatogram Immersion Device) and the results converted into a digitalised imagine. Software can memorize the plate and convert the analysis into a sequence of peaks or a 3D sequence of tracks at different wavelengths or make quantitative calculation.

Enhancement of sensitivity: current methods developed by Camag scientists can quantitatively detect the dyes down to 2ppm. Thus, manual operations are limited to the preparation of the samples, to the movements of the plate in the sequence of devices, as well as to the use of the proper software in the computer.

THE HPTLC WORLD

Interest in HPTLC applications remained for long time a niche curiosity, used in some cases to perform a high quality planar chromatography of too complex mixtures. The feature changed completely with the introduction of the computer-controlled devices. The situation is evidenced by the scientific papers dedicated to HPTLC and published in the last years: in 2010 they arose to more than 100, respect to the previous insignificant numbers. The year 2011 is even more promising (Table 1 and Fig. 1).

The geographic distributions of the provenience of the papers are also interesting: developing countries are the majority, with a clear predominance of India (Fig. 2). This is another evidence that the scientific gap of developing countries is rapidly decreasing and is of different attention for novelties. This is another indication that globalisation is not so far. This is another clue that HPTLC just started its pathway to embark on an important future.

The reasons of the boom are essentially technological. The computerised instrumentations, like those performed by Camag, changed radically HPTLC, maintaining and improving visibility, easiness and quickness of TLC, but adding robustness, accuracy and reproducibility, in other words turning a material

TABLE 1 Number of papers concerning HPTLC as reported by SciFinder using the keyword "HPTLC analysis".

Year	Published papers
2008	2
2009	5
2010	107
2011[a]	19

[a]*Up to January 2011.*

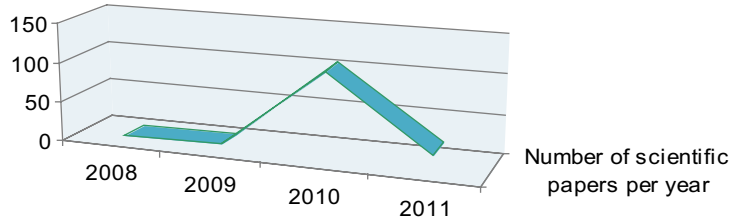


FIGURE 1 Evolution of scientific papers dedicated to HPTLC. 2011 up to January.

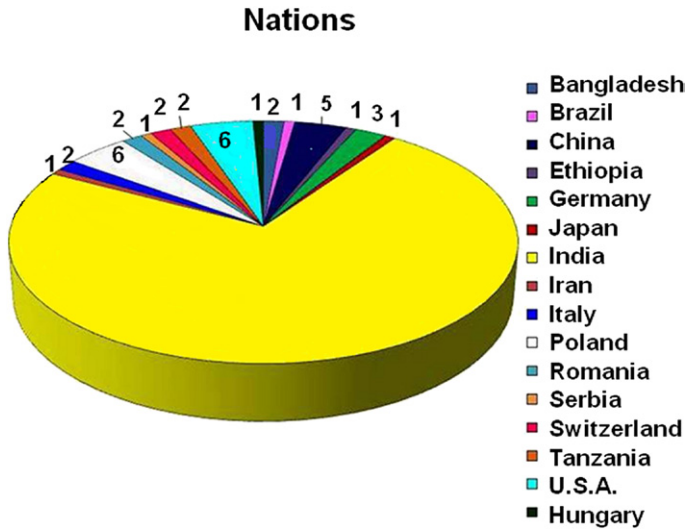


FIGURE 2 Geographic distributions of the provenience of the papers dedicated to HPTLC. India accounts for 64% of the total number.

detail into a complete and reliable method, able to rapidly catch the attention of the analysts [10].

A second decisive aspect is the insistent effort to transform HPTLC into a complete technique tailored to face the phytochemical problems. Thus, the densitometric approach allowed the solution of one of the TLC deficiencies: bi-dimensional visualization and quantification. The 3D plot based on multiwavelength scan of the plate gives an impressive look: in multiwavelength densitometry each track is scanned with up to 31 selected individual wavelengths of the electromagnetic spectrum, from UV to visible. Plotting against Rf and adsorption values generates a three-dimensional plot of the tracks of the plate, best evidenced by different colours for each track or wavelength (Fig. 3).

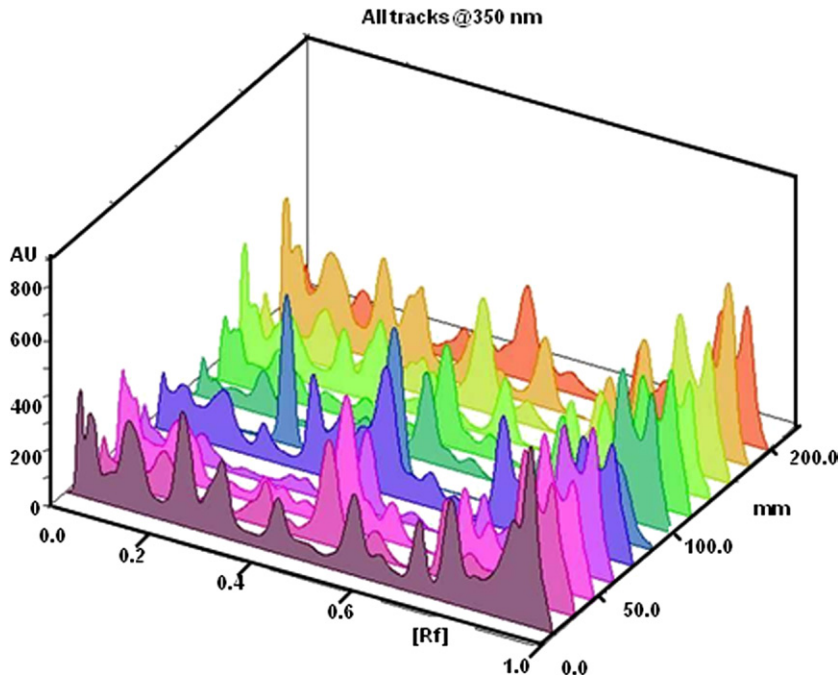


FIGURE 3 Example of 3D densitometric chromatograms at 350nm. Each profile corresponds to a track where the intensities of the spots can be appreciated and measured in order to obtain a quantification of the separated substances.

The New Epiphany of Planar Chromatography

Yes, but where is HPTLC going? Let have another look to the published papers. About 1/3 are dedicated to standardization and identification of pharmaceutical products and drugs, but more than 2/3 are focused on studies of medicinal plants and natural products mainly in the pure phytochemical tradition. This is confirmed by the distribution of papers among the different classes of natural substances: terpenes are predominant (33%), followed by phenols (26%) and alkaloids (15%), whereas primary metabolites account only for 10% (Fig. 4).

However, it is possible that this situation will rapidly change. As already for TLC, the importance of HPTLC will not be within the research, but in the applications, according to the requirements of the moment. Therefore, the aim of this paper is to show applications of HPTLC in the food supplements sector, in order to offer a picture of a part of the present and a glance at the near future.

HPTLC Fingerprint and Botanicals

The full power of HPTLC comes from the proper use of each device in an integrated system [8]. Frequent goal of HPTLC is the fingerprint, as authentic maker

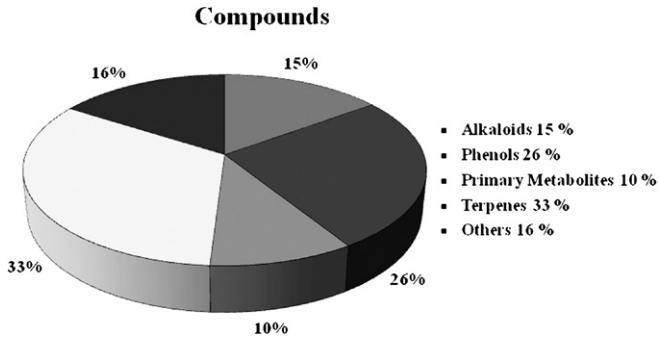


FIGURE 4 Distribution of published papers among the different classes of natural substances as reported in the scientific literature.

of the biological complexity. A fingerprint is the individual chromatographic track representing, as near as possible, a mixture of organic substances. By the fingerprint approach, it is not only possible to obtain a proper identification of the plant material, but also to determine and accept the limits of the biological changes. This approach can be pursued also by other techniques [11–13], but HPTLC usually prevails for the quantity of information that can be easily obtained.

In the tradition of planar chromatography usually the identification of a substance is obtained by comparison with selected standards and in this analytical step the efficiency of the separation that can be obtained in HPTLC is clearly crucial (Fig. 5). In the multiwavelength chromatogram the position of the peak maximum, as a consequence of the retention of the substance, can be used for identification in qualitative analysis and, also in a crowded track, the identity of a spot with the standard can be selectively visualised and evidenced, as well as confirmed by the direct analysis of the proper UV spectrum of the spot. Another improvement of this application is that the intensities of peaks can be used for quantitative determinations.

After integration of the chromatogram and quantification to a reference standard, the height or the area of each peak can be measured by a detector

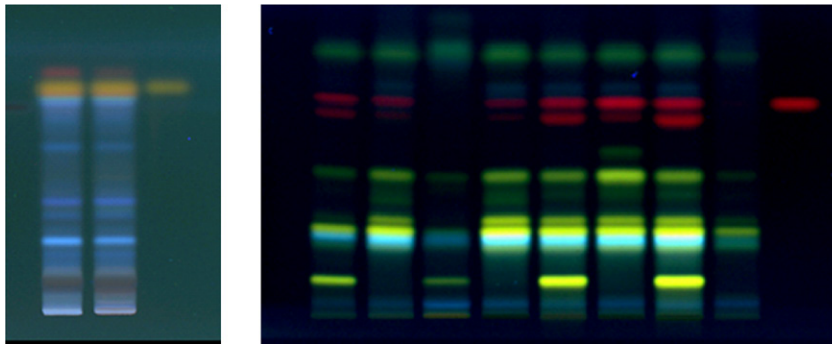


FIGURE 5 Examples of clear identification of reference standards in complex mixtures: on the left plate, tracks: 1, 2, Senna extracts; 3, rhein; on the right plate: 1–8, St John's wort extracts; 9, hypericin.

responding to a charge of mass per unit of time, as in other techniques, e.g., HPLC [9,14] and NMR [11,13]. Also in this case, the presence of noise and asymmetry of the peak (tailing or leading), that means quality of the plate, are important. Other parameters concern the wavelength that must be selected at the highest absorption of the sample or at least at the maximum to ensure sensitive detection.

Benefits from HPTLC

It is important to reflect on advantages of HPTLC in order to understand its possible applications. Each step of the analysis must be paired with the appropriate device and referred to the main goal, which is the chromatogram as a plate filled by sequences of evident spots, dark, coloured and fluorescent. Many samples, up to 72 (usually 20) on a 20×10cm HPTLC plate, can be analysed side-by-side in the same conditions by a rapid parallel screening, also including the use of selected standards. The improvements in the quality of the planar chromatography obtained by the use of HPTLC approach and proper devices are evident and can be discerned step-by-step, starting from the deposition. Fig. 6 shows the

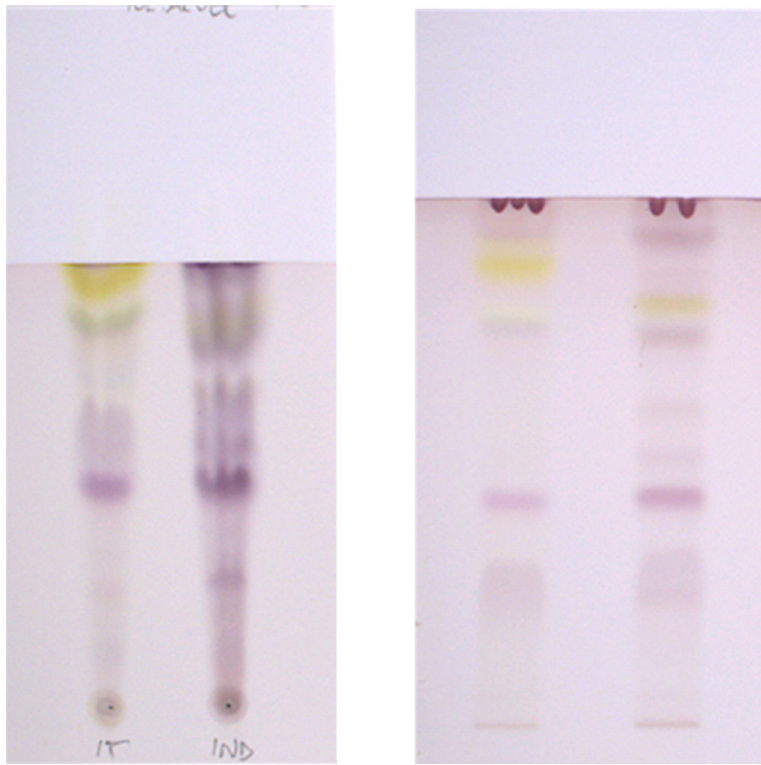


FIGURE 6 TLC plates: comparison between manual (first plate) and instrumental applications (second plate) of the same plant extracts.

comparison between manual and instrumental application of the same extract on a simple TLC plate. The improvements using this simple change are evident, although the spots are clearly the same. The used instrumentation has been developed to face the problem of complex mixture of natural products that means controls of the performance conditions, high quality separation and best visualization of the plate. In any case, each analysis must be considered as a single problem and several parameters, e.g., elution solvent systems, concentrations of the samples, etc., must be carefully considered. Many preliminary tests must be performed in order to achieve the adequate results.

After development and before derivatization, the same plate can be visualised in different ways, using lights at different wavelengths, like white light, UV254 and UV366nm, followed if necessary by particular detection using a derivatization with selected reagents (Figs. 7 and 8). The multiple detections can give a lot of information and the visualization can be optimized and stored at the best possibility by scanning and acquisition in the computer memory. Once in the computer the images of the tracks obtained using different visualizations can be compared with the database containing the data of plates obtained in variable times or in variable places (Figs. 9 and 10). A plate made in Rome can be easily and immediately compared with another one made under the same conditions and with the same apparatus at New Delhi.

Figures 11 and 12 show the identification method of a reference standard in complex mixture using the Videoscan conversion. The interpretation of the

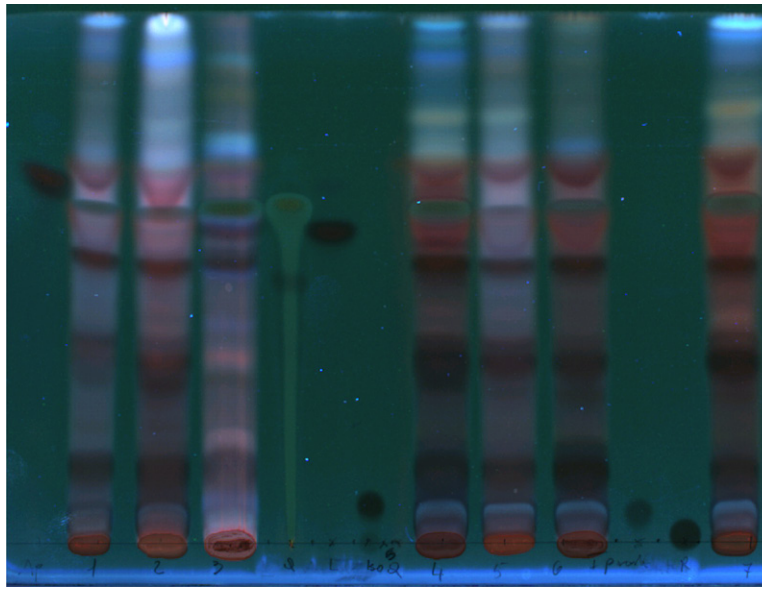


FIGURE 7 TLC analysis, obtained by manual application and development in a tank, of St John's wort extracts (Tracks 2–4; 8–10; 13) and selected flavonoids standards (Tracks 1; 5–7; 11, 12).

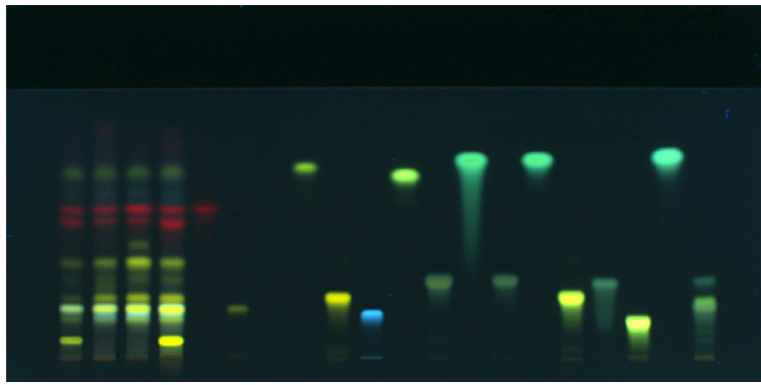


FIGURE 8 HPTLC analysis of the same extracts of Fig. 7 using instrumental method and controlled conditions. Tracks: 1–4, St John's wort extracts; 5, hypericin; 6–19, flavonoids standards.

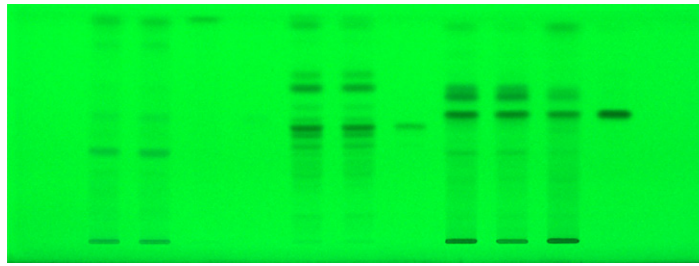


FIGURE 9 Visualization of a HPTLC plate at UV254nm. Mobile phase: ethyl acetate, acetone, formic acid, water (15:9:3:3). Tracks: 1–3, extracts of *Echinacea* spp. with different extraction methods and times; 4, chlorogenic acid (not evident); 5 and 6, extracts of liquorice with different extraction times; 7, monoamine glycirrinate; 8–10, *Harpagophytum procumbens* with different extraction methods and times; 11, harpagoside.

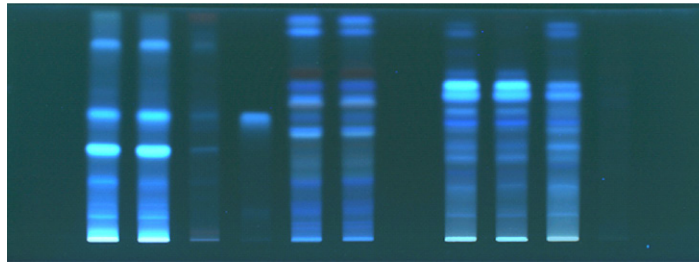


FIGURE 10 Visualization of the same plate of Fig. 9 at UV366nm. Glycirrinate (track 7) and harpagoside (track 11) are not visible in these conditions, whereas chlorogenic acid (track 4) is now evident.

HPTLC results is immediate and very simple. Any type of experience or specific knowledge is not necessary to understand the tracks of a plate, especially in the case of comparison. The operation of the plate is easy and it does not need a dedicated operator with years of experience, a normal knowledge for the use

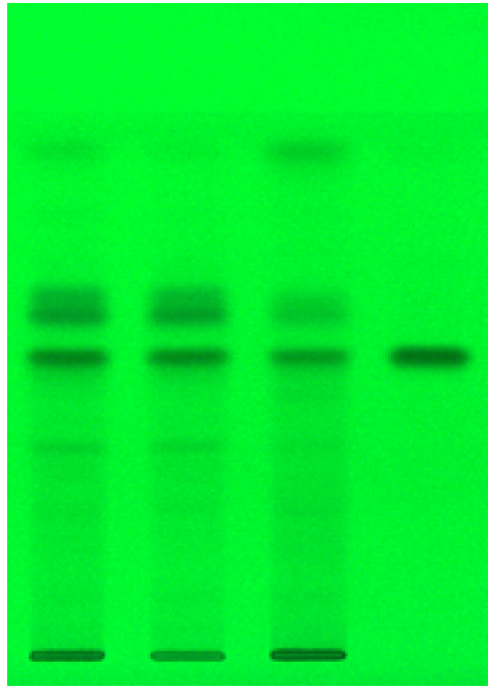


FIGURE 11 HPTLC fingerprints of extracts of *Harpagophytum procumbens* corresponding to tracks 8–10 of Fig. 9 in comparison with the selected standard (harpagoside). Mobile phase: ethyl acetate, acetone, formic acid, water (15:9:3:3). Derivatization: anisaldehyde. Visualization: white light. Tracks: 1–3, *Harpagophytum procumbens* extracts at different concentrations; 4, harpagoside.

of common software is enough. This is in contrast with the dominant trend in chemical analysis.

Unfortunately, in the last years analytic chemistry evolved in a persistent search of high specialization, enormous cost and difficulties for ordinary people to understand what is going on. A pernicious tendency is common to many applications of chemistry knowledge. Among the consequences of a precious insistent specialization, there is the lost capacity to obtain a total glance of the composition. The real risk is the capacity to check the presence of minimum quantity of a substance and to be blind in detection of other substances present in high quantity.

One Picture is Worth Ten Thousand Words

Several parameters must be considered in quality control analysis using HPTLC. Flexibility is derived by the multiple parameters that can be used to affect the separation. Although universally silica gel is the first choice and a pair of solvent systems can be adopted to obtain engaging results in any case, a wide choice of mobile phases can be combined with different stationary phases. Change of the

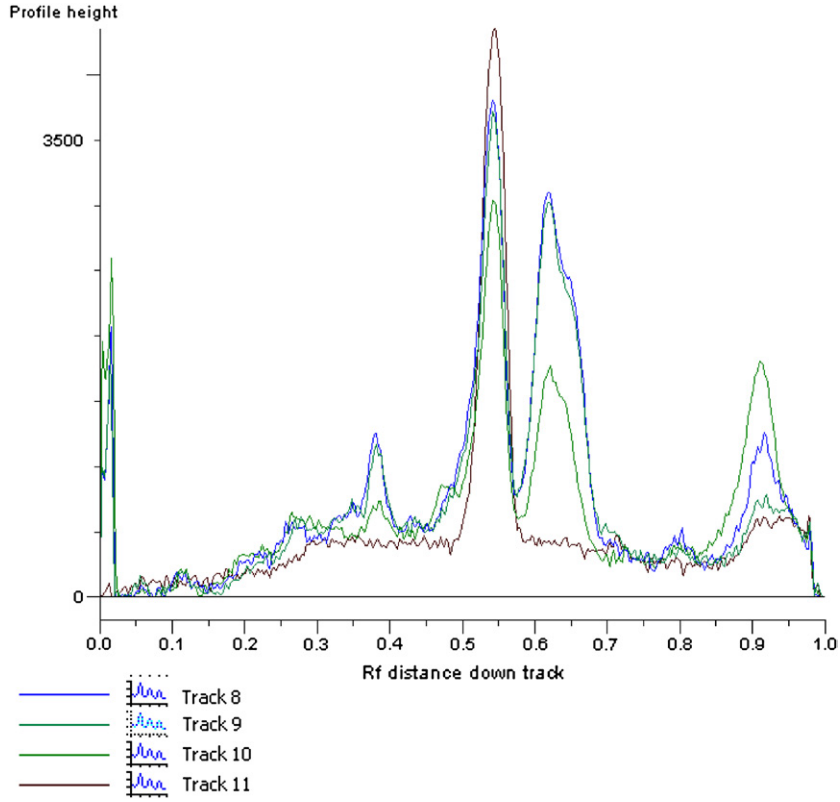


FIGURE 12 Visualization of plate of Fig. 11 using the Videoscan conversion. Spots are converted in peaks and the tracks converted in sequence of peaks. The harpagoside standard is the track 11 and its presence is confirmed by the identity of the peaks of the other tracks at selected wave. Intensities of peaks can be used for quantitative determination.

mobile phase allows adapting the chromatogram to different classes of natural substances and their different polarities and chemical functionalities, as usual in a herbal extract and is very useful to confirm the identity of constituents against selected standards.

Quality can have a commercial value when it can be visualised and materialised in understandable data. The polychromatic plate exerts a beautiful appeal and owns an intrinsic value. The main target of HPTLC is in practical applications on herbal raw materials and their derived products, in order to reply quickly to simple but economically important questions.

The most common case concerns the identity of the used herbal raw material, generally present in the form of an extract. In this case, to confirm the identity, the analysis can use the comparison with selected standards and/or another extract whose composition is known. Thus, in the case of an extract of leaves of Passion

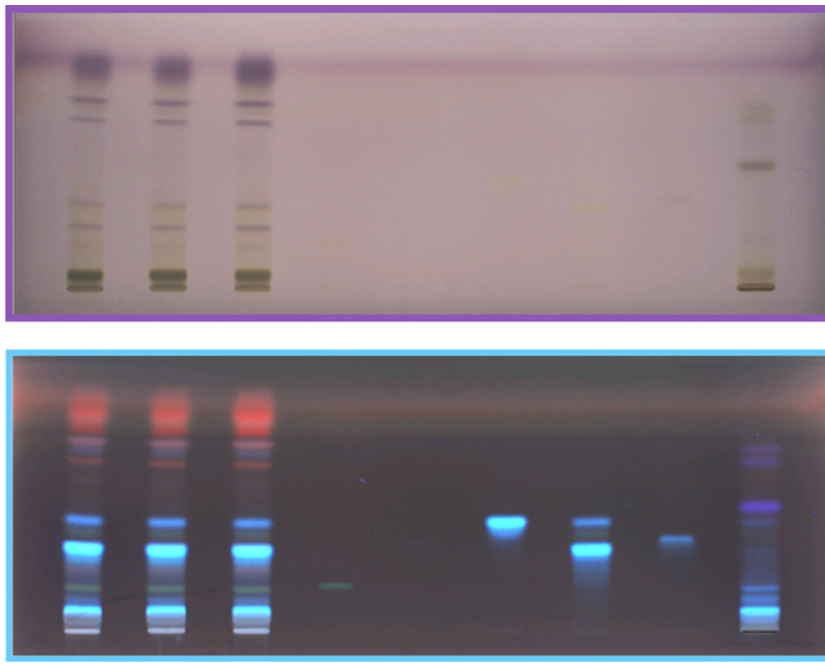


FIGURE 13 Analysis of extracts of Passion flower using the HPTLC fingerprint, in comparison with flavonoids standards and a reference extract. Mobile phase: THF, toluene, formic acid, water (16:8:2:1). Derivatization: anisaldehyde. Tracks: 1–3, methanol extracts of Passion flower; 4, rutin; 5, hyperoside; 6, vitexin; 7, isovitexin; 8, chlorogenic acid; 9, hydroalcholic extract of Passion flower. Plate on the top, visualization: white light. Plate on the bottom, visualization: UV366nm. Isovitexin standard resulted very similar, also in the impurities, to the central spots of tracks 1–3, whereas track 9 presents some additional spots, probably due to a different extraction method.

flower, *Passiflora incarnata*, the fingerprint of the extract was compared with several flavonoids and a known extract, owing to the complexity and both the information resulted were useful (Fig. 13). The use of reference harmane alkaloids, considered among the most important active principles of Passion flower, was discarded because of their great variability, either in the plants or in the extracts.

Using a longer time for the extraction is the result improving? The fingerprint of two extractions at 12h and 21 days shows the absence of evident differences in some studied cases (Fig. 14).

After the extraction process, does the cake still contain interesting compounds and perhaps could a further extraction be useful? Are the products obtained by selected plants and with a special method of extraction different and better against other similar market products? The plate reported in Fig. 15 evidences the differences in the fingerprints of market products obtained from neem, *Azadirachta indica* (see later for further details).

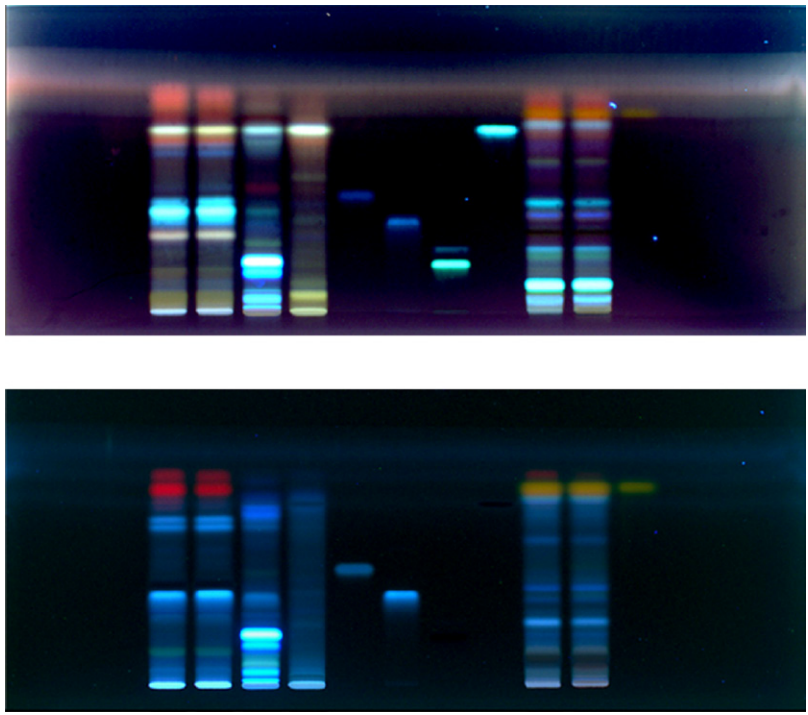


FIGURE 14 HPTLC analysis of *Cynara scolymus* and *Cassia senna* extracts obtained with the same apparatus but at two different times, compared with specific standard. Mobile phase: THF, toluene, formic acid, water (16:8:2:1); Derivatization: anisaldehyde. Plate on the top, visualization: UV254nm. Plate on the bottom, visualization: UV366nm. Tracks: 1,2, extracts of artichoke after 12h and 21 days, respectively (differences are minimal); 3,4, extracts of artichoke from the market; 5, cynarine; 6, chlorogenic acid; 7, rutin; 8, luteolin; 9,10, extracts of senna after 12h and 21 days, respectively (differences are minimal); 11, rhein.

With one HPTLC plate we can answer the most common questions of the herbal market. Is it the right species? Which is the best among the proposed raw materials? Is our extract comparable or even better than those in the market? Did the extract remain stable during the storage? Are there remaining traces of the solvent? Is any adulterant present? Necessary, each of these questions means money. Usually, each of these questions needs a quick and simple reply. Clearly, each of these questions implies quality and its determination by analytic controls. In planar chromatography, quality is inherent with the quantity of spots in the track and their evidence in the visualization process that means efficiency, the peculiar strength of HPTLC.

This efficiency is reported in these and other examples concerning analyses of extracts obtained from industries, commercialised with the same name, like the plate reported in Fig. 16.

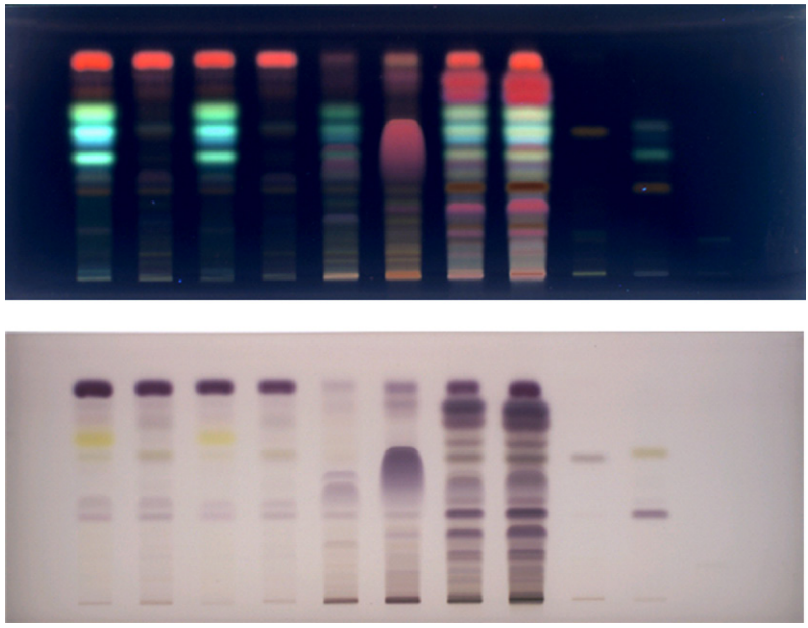


FIGURE 15 HPTLC analysis of different neem (*Azadirachta indica*) products. Mobile phase: toluene, ethyl acetate (4:6). Derivatization: anisaldehyde. Plate on the top, visualization: UV366nm. Plate on the bottom, visualization: white light. Tracks: 1, neem oil marketed in Italy extracted with ethyl acetate; 2, neem oil marketed in India extracted with ethyl acetate; 3, neem oil marketed in Italy; 4, neem oil marketed in India; 5,6, neem cakes extracted with ethyl acetate; 7,8, neem cakes of tracks 5 and 6 furtherly extracted with ethyl acetate and concentrated; 9, nimbin; 10, salannin; 11, azadirachtin A. The red spots visible at UV366nm are mainly fatty acid constituents (determined by isolation and NMR).

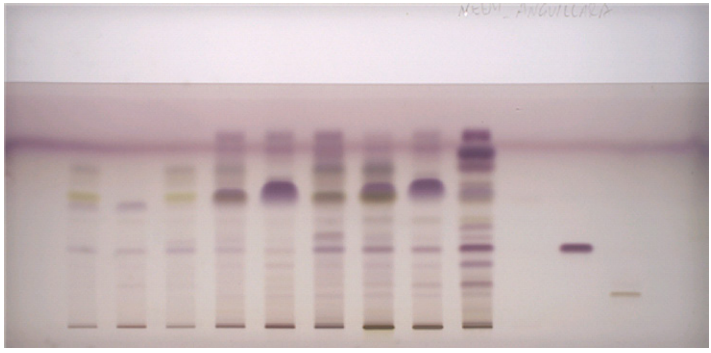


FIGURE 16 HPTLC analysis of commercial neem cakes products. Mobile phase: toluene, ethyl acetate (4:6). Derivatization: anisaldehyde. Visualization: white light. Tracks 1–9 neem cakes from different producers or sellers, 10, nimbin; 11, salannin; 12, azadirachtin A. The different compositions are evident in particular for the lipidic constituents, e.g., main spots at high Rf values in tracks 4,5,7,8 that are practically absent in tracks 1–3. The pink line near the front line is due to the absence of a pre-cleaning of the plate. Therefore, to avoid this effect before the analysis, the plate must be first eluted with methanol and carefully dried.

Biodiversity Consequences

Variation is a biological characteristic and the advantage of victorious competitors in life evolution struggle. Accumulation of secondary metabolites in plants is influenced by an enormous quantity of environmental factors, as well as by harvesting and post-harvesting conditions, like herbal processing, storage conditions, extraction processes and others.

The result is great difference in concentrations of constituents and problems with regulatory authorities. Again, the temptation is to treat living organisms as biofactors for production of useful molecules. Herbal medicines continuously face quantitative problems, owing to the final aim of obtaining the standardization of herbal extracts. Most of commercial herbal raw materials would be kept out of the market by too narrow and rigid quantitative limits. On the other hand, the absence of any quantitative reference will have a negative impact on the quality of the commercial raw materials and their derived products. Although diversities in fingerprints of the same species can be observed, this usually affects the areas of the peaks and not their presence.

Pre-requisites are the authentication of raw materials as well as the presence of specific compounds considered important for the activity. Both targets can be achieved by the HPTLC fingerprint.

HPTLC fingerprint can be used in direct revealing of chemical profiles of the mainstream of market products. Lots of the same product can be compared in the same plate and quantities assessed by densitometric inspection. Applications of HPTLC fingerprint are here reported in the steps of botanicals birth and development: (a) selection and identification of raw materials; (b) extraction and transformation; (c) checking of the final product; and (d) adulteration and falsification.

Vegetal Body Identification

Regulatory documents on medicinal plants, including Pharmacopoeias [15], are in persistent search of methods for identification and determination of herbal matrices. In a global market, several new species are already used for several years, but they are still waiting a legal recognition; meanwhile other plants are used instead of or confused with the traditional ones. The HPTLC fingerprint approach can give an easy differentiation of the different species, certifying in some cases also the geographic origin, as reported in the HPTLC volume of the Chinese Pharmacopoeia [16] (Fig. 17).

CASE STUDIES

Variations at Species Level: The St John's Wort Case

The study of St John's wort, *Hypericum perforatum*, shows the evidence of variation in secondary metabolites production by plants of the same species. In this

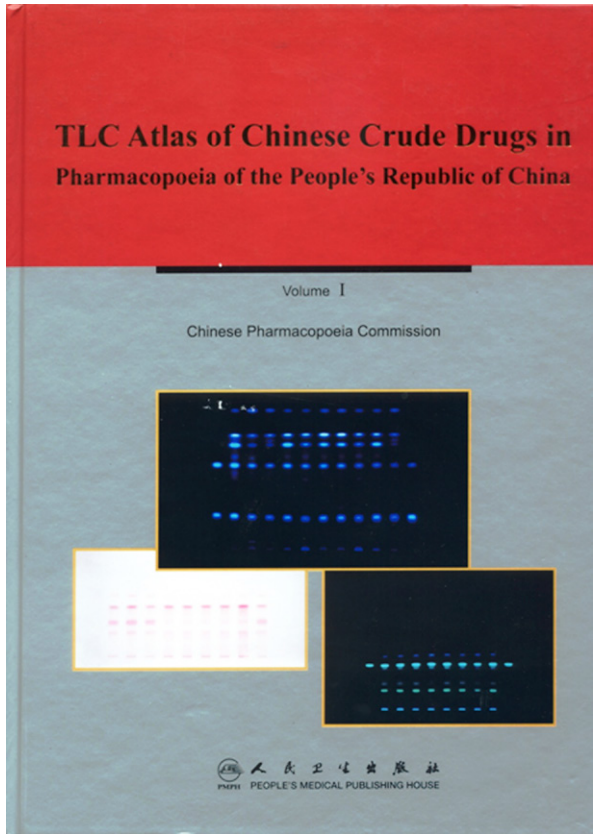


FIGURE 17 The volume of the Chinese Pharmacopoeia reporting the HPTLC fingerprints of the most important herbs of the Chinese medicines market.

case, the use of standards is crucial. Raw materials, consisting of fresh aerial parts collected in different regions of Italy in the flowering period, were examined by HPTLC fingerprint. The plate showed similar profiles of methanol extracts of fresh flowering plants, as the evidence of the same basic composition, typical of the species, but variances in intensities are equally clear. In particular, the analysis needed to be completed by the comparison with several standards (Fig. 18). The HPTLC showed in all the populations the presence of several flavonoids, as well as that of hypericin as an evident red spot, coupled with that of pseudohypericin at lower Rf. In Italy, nowadays a definite rapport between hyperforin and hypericin is asked for marketing food supplements containing St John's wort, but this is clearly against the natural variance of this species, as evidenced by the HPTLC analysis. In the analysis it was included a commercial extract, among the most used in Italy, whose fingerprint evidences a clear difference with the other extracts including a strong diminution of hypericin concentration.

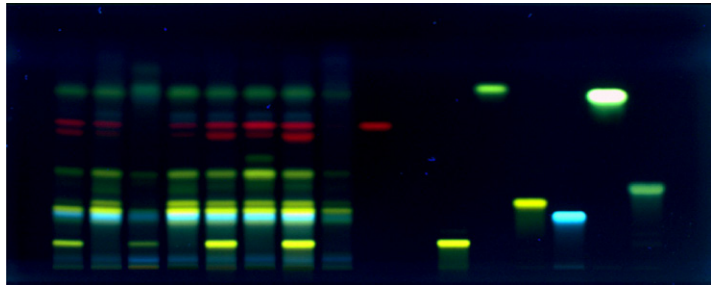
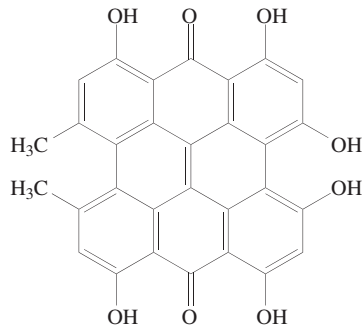
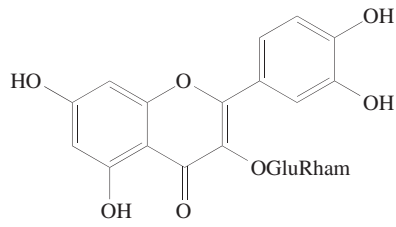


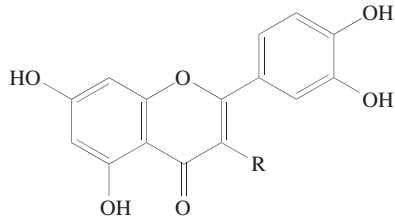
FIGURE 18 Comparison among different populations of *Hypericum perforatum*. Mobile phase: formic acid, water, ethyl acetate (10:5:85). Derivatization: the layers were treated with a solution containing the Natural Product Reagent (NPR) (1g diphenylborinic acid aminoethylester in 200mL of ethyl acetate), dried in the open air and then dipped into Macrogol reagent (1g polyethylene glycol 400 in 20mL of dichloromethane). Visualization: UV366nm. Tracks 1,2, 4–8 fingerprints of St John's wort extracts collected in different Italian regions at the same vegetative period (flowering), 3, fingerprint of a commercial extract; 9, hypericin; 10, hyperoside; 11, rutin; 12, quercetin; 13, isoquercetin; 14, chlorogenic acid; 15, luteolin: 16, apigenin. The presence of hypericin is evident as a red spot (the lower coupled red spot is pseudohypericin), as the differences in its concentrations are also evident. Other differences concern the presence and quantities of some flavonoids.



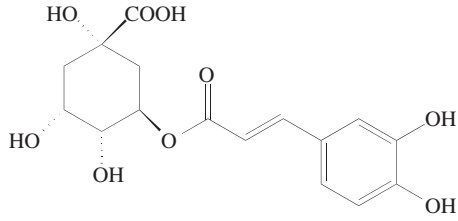
hypericin



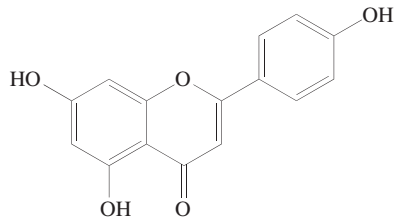
rutin



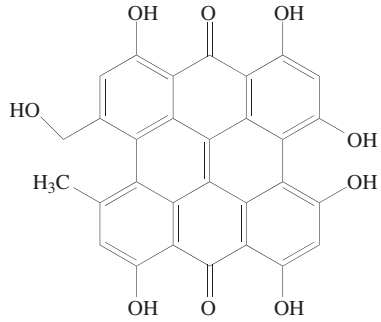
R = H luteolin
R = OH quercetin
R = O-glucose isoquercetin
R = O-galactose hyperoside



chlorogenic acid



apigenin



pseudohypericin

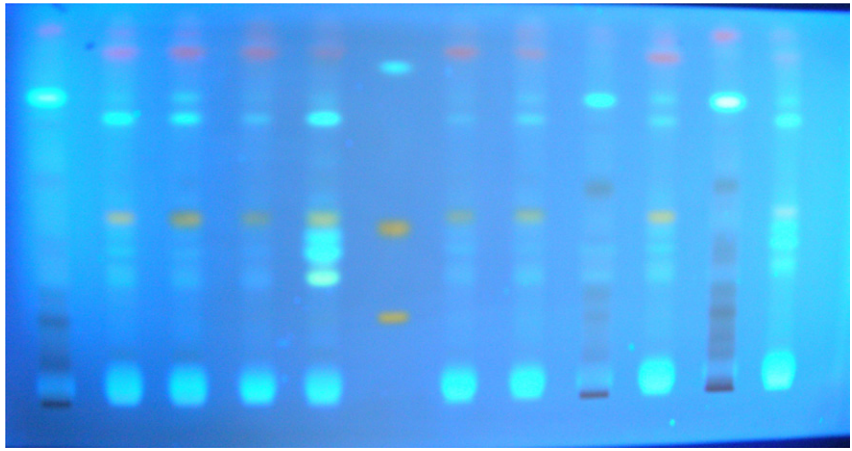


FIGURE 19 HPTLC of *Equisetum* spp. Mobile phase: ethyl acetate, formic acid, water (82:9:9). Derivatization: NPR and Macrogol. Visualization: UV365nm. Tracks: 1,11, *E. maximum*; 2,5,7,8,10, *E. arvense*; 3,4, identified as *E. arvense* on the basis of the fingerprint; 6, caffeic acid, hyperoside and rutin; 9, identified as *E. maximum* on the basis of the fingerprint; 12, *E. arvense* CTS, from European Pharmacopoeia.

Variations at Genus Level: The *Equisetum* Case

Other similar studies were focused on the genus level, for products containing horsetail, *Equisetum* spp., whose stem extracts are well known for their diuretic and haemostatic properties [17–19]. According to the European Pharmacopoeia [20] only *Equisetum arvense* should be used in case of urinary tract inflammation, but other species such as *Equisetum maximum*, *Equisetum ramosissimum* may be present on the market, including the toxic *Equisetum palustre*. The HPTLC fingerprints of methanol extracts of 10 samples of *Equisetum* raw materials from different manufacturers and countries of origin were examined. They were compared with the *E. arvense* stem reference standard of Pharmacopoeia (*E, arvense* CTS) and with the chemical reference standard solution of the European Pharmacopoeia, which contains caffeic acid, hyperoside and rutin. Five samples were in accordance with that of the *E. arvense* CTS, as well as two other samples, generically named "Equisetum". Two other samples, named *E. maximum,* gave fingerprints valid for the specific fingerprint of *E. maximum*, as well as one sample, generically named "Equisetum" (Fig. 19).

Differences in fingerprints of the same species were mainly quantitative and are more readily discernible in densitogram, thanks to the three-dimensional visualization (Fig. 20).

The same samples were examined by HPLC, obtaining similar and comparable results.

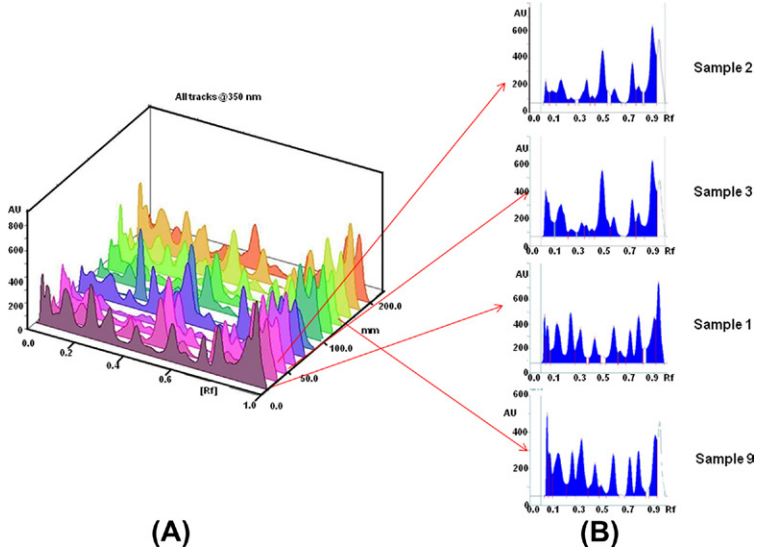


FIGURE 20 (A) 3D densitometric analysis of samples reported in Fig. 19. (B) Typical fingerprint of "Equisetum".

Control of Quality in the Processing Level: The Neem Case

Qualitative assessment of raw materials must be beyond the mere identification and is usually linked to the chemical composition.

In particular, we examined the composition of several products marketed by the name of neem cake. Neem tree (*A. indica*), also known as nimba, native of India, features a long traditional utilization in agriculture and Ayurvedic medicine, but currently there is a huge amount of products obtained from this multipurpose tree, due to the medicinal, anti-fungal, anti-bacterial and insecticide properties. The main use of neem is not directly medicinal, but in some way we are in the same context, since insects transmit serious diseases like malaria, filariasis, dengue, haemorrhagic and yellow fever, that means millions of deaths every year. After a long devastating use of chemical insecticides, whose adverse effects on environment, operational cost and community acceptance are evident, numerous plant products are in use or have been reported as one of the best alternatives for killing larvae or adult mosquitoes or as repellent for mosquito biting. Neem, identified by WHO/UNEP1989 as an environmentally powerful natural pesticide, is considered to be one of the most promising trees of the twenty-first century for its great potential in pest management, environment protection and medicine. There are about 14 million neem trees growing only in India and the plant is adapted to subarid and subhumid areas of tropical and subtropical zones, and therefore widely spread and introduced in many countries, from Cuba to Australia.

Reported activities of neem against insects are antifeedant, ovicidal, fecundity suppression, growth regulation and repellence, but the treasures of neem are in the

environmental care. Control of crop pests has led to the adoption of eco-friendly natural insecticides, whose minor pesticidal efficacy is balanced by environmental safety and public acceptance. When produced with respect to their natural origin, neem products have low toxicity to birds, fish and mammals, and they are less likely to induce resistance due to the multiple mode of actions, including the weakening of the cuticle defence system of larvae, because of the richness of the phytocomplex. In addition, most of the active components are decomposed by exposure to solar light. The most important commercial product is the oil, obtained by several methods from the seeds. Neem expeller oil is manufactured by cold pressing neem kernels from handpicked and cleaned neem fruits and seeds. Neem oil acts as an insecticide and protects plants and crops against pests.

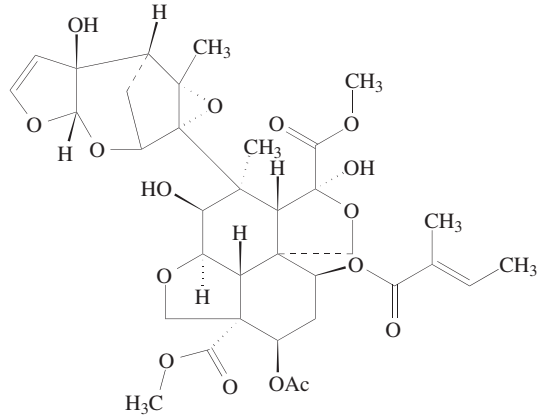
Neem can be considered the Mt. Everest of the analytical study. Neem seeds contain more than a hundred of biologically active compounds and at least the same number of other important constituents was identified. However, other constituents are actually found. Among active principles as botanical insecticides, extracted from neem, several complex tetranor-triterpenoid limonoids must be considered responsible for both antifeedant and toxic effect in insects: azadirachtin A, the predominant component, azadirachtin B, nimbin, salannin and related compounds, that have proven to be effective in the control of agricultural pests [21]. The most famous constituent, azadirachtin, evidences 7 cycles, 16 stereogenic centres and an exceptional quantity of oxygen, causing the synthesis approach an enormous problem, only recently elegantly solved. Other considerations must be assigned to the strong natural variability and the availability of the standards, considering the difficulty of separation and the low stability of these compounds.

There is a huge scientific literature, which reveals both the antifeedant and the physiological effects of these substances. Reported contents of azadiracthin A in the different formulations of neem expeller oil greatly change from 300 to 2000ppm. Neem cake is the raw material derived by cold pressing neem kernels. There is an increasing interest in the exploitation of exhausted materials, but their validation needs great technologic and scientific efforts. Neem cake is commercially important for its use as fertilizer, as well as potential insecticide [22].

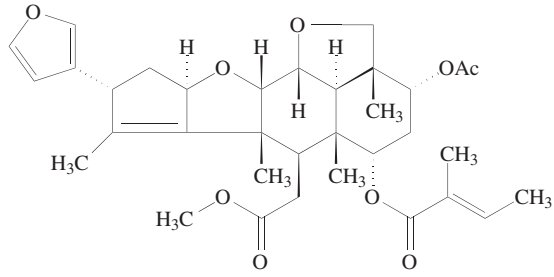
By the HPTLC the analysed neem cakes are different, depending upon the extraction process used, the different origins and the method of commercialization. In particular, the content of fatty constituents appeared highly variable, in some cases predominant and highly influencing chemical–physical properties as well as possible utilizations. The HPTLC can show either the identity of the plant and the presence and quantities of different constituents, including the active nor-triterpenes (Figs. 16 and 21).

As a matter of fact, the HPTLC study of neem cake products (Figs. 21 and 22) showed the different presence of fatty acid constituents, clearly visible at UV366nm as red spots, whereas nor-triterpenes, due to their oxygenated nature, are confined at lower Rf values. To evidence the salannin it was necessary to use the derivatization and the inspection at the white light. However, the analytic attention was focused on the middle of the plate, where three spots, mixed to several spots related

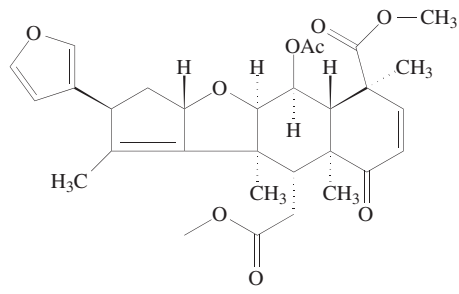
to fatty constituents, were very evident at the UV366nm in tracks 14–16. These compounds should be considered the markers of the neem cake products, albeit they can be overlapped by fatty acids as evident in several tracks (Figs. 21 and 22).



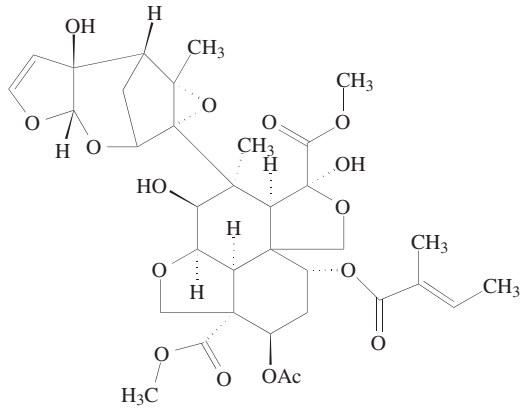
azadirachtin A



salannin



nimbin



azadirachtin B

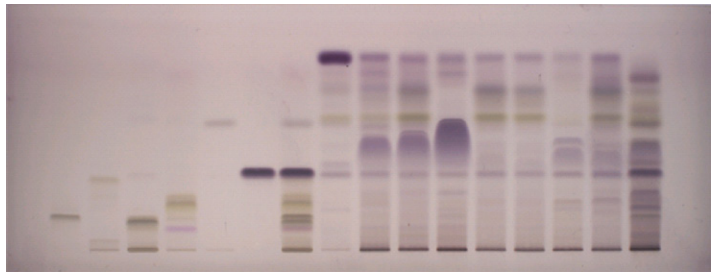


FIGURE 21 HPTLC fingerprints of neem products (on the right side) and selected standards (on the left side). Mobile phase: toluene, ethyl acetate (4:6). Derivatization: anisaldehyde. Visualization: white light. Tracks: 1, azadirachtin A; 2, azadirachtin B; 3, azadirachtin D (11-epi-azadirachtin A); 4, 11-deoxy-azadirachtin A; 5, nimbin; 6, salannin; 7, the previous standards all together; 8, neem oil marketed in India; 9, methanol extract of neem cakes from India; 10–15, methanol extract of commercial samples of neem cakes; 16, ethyl acetate extract of neem cake sample 14.

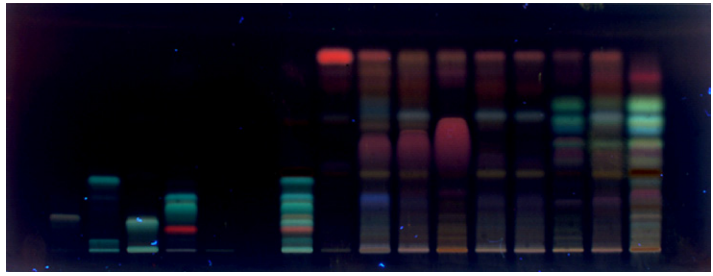
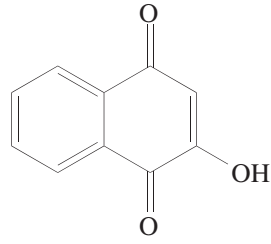


FIGURE 22 The same tracks of Fig. 21 visualized at UV366nm. In particular, by separation and NMR identification the main red spots evident in the middle of the tracks 9–11 resulted as a mixture of fatty acids.

Into the Market: The Henna Case

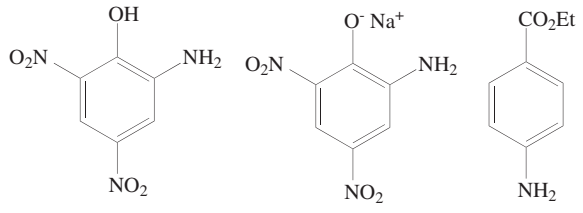
To tell about henna, it is necessary to start from the beginning of Phytotherapia. The oldest example of Materia Medica is contained in the Ebers Papyrus. A total of 20 metres of papyrus written in the ancient Egypt about 1500 BC, but it is believed to have copied from earlier texts, perhaps dating as far back as 3400 BC. Among the some 700 magical formulae and remedies, a detailed coverage was dedicated to henna as tribute of the complexity of this product and its plant, *Lawsonia inermis*. Seven types of henna were catalogued according to the origin. KPR, the ancient Egyptian name, "from the north", "from the fields", "from the marshes", were specifications of quality, since the soil and moisture affect henna composition, in particular in the content of the most important constituent, lawsone (2-hydroxy-1,4-naphtoquinone). Moist, fertile, cool conditions produce low lawsone content, the contrary of dry, hot, iron-bearing soils. Thus Ebers Papyrus gives us the first indication for the quality of product: in fact, henna can have different contents of lawsone in different conditions of soil and moisture, with variations from 0.17 to 0.60wt%. The Papyrus contains another indication: "knots of henna", "thorns of henna", "henna grass" means parts of the plant specific to age and growth cycle and, as reported in analytical quantifications, lawsone content varies according to the time of collection. The third indication is related to the treatments, like the mixture with other herbs, mainly aromatic, and/or the addition of ingredients to create a mildly acidic paste or the adding olive oil or resin to handle the paste. These treatments could be related to the different medicinal use, like in fungal or bacterial infections. In conclusion, the Ebers Papyrus contains the current observations on the differences in henna products according to the growing areas, the used plant parts and the addition of other ingredients. These indications are still at the basis of any careful analytical study. In other words, the analysis of a botanical can be completed by a series of information and knowledge useful to tailor the work.



lawsone

From ancient Egypt and neighbour countries, the use of henna has continued for thousands of years in Arabian countries and nowadays it is expanded all over the world. The main use is cosmetic, to dye hair and nails and for decorative body painting. However, the use of hair dyes has dramatically increased during the last decades. Several types of henna are on the market: commercial red

henna is based on *L. inermis*, black henna on *Indigofera tinctoria*, neutral henna on *Cassia obovata* and blond henna on *C. obovata* with addition of several other plants. The colouring action of red henna is usually due to lawsone, present in the leaves together with its glucosides (hennosides). In the dried leaves, besides lawsone (0.5–2%), other compounds are also present, co-occurring in the activity as well as in characterization of the raw material, like polyphenols (flavonoids, gallic acid) and glucides. Henna is considered safe, although one study claimed that the application can induce a severe haemolytic anaemia and may contribute to neonatal hyper bilirubinemia. However, a real toxicity for henna should be excluded on the basis of investigations on large populations and on consumers or professional exposures. The lawsone position is different: its content has to be checked and essayed for its potential toxicity because some cases of renal damage and bladder cancer have been reported. Thus, in EU the content of lawsone in cosmetic preparations must be avoided and limited to 1.5% in products based on Henna [23] and declared in the label. Furthermore, the Scientific Committee on Cosmetic Products and Non-Food Products declared that no safe threshold for lawsone can be established. In any case, there is an interest in determining the lawsone content in cosmetic agents, owing to the wide variation of quality. Meanwhile the demand of henna is increasing, at the same time the number of henna powders on the market is growing, including the case of adulterations with other dying substances to reinforce the activity, like the adding of picramate and picramic acid, whose concentration in cosmetic products must be below 0.1% according to the Cosmetic Ingredient Review. Another example concerns the paraphenylendiamine, present in the black henna or henna, as a possible cause of cutaneous irritations and burns. However, most of the quality problems are related to plant ingredients: henna can contain fruits and twigs, as well as parts of other plants. The aim of the addition of other plants or natural or chemical substances could be the modification of the hair colour or to improve the penetration or the fixing of the dye or to reinforce the hair colour or to improve hair-protection, effects given by a lot of associated additives, from keratin to heavy metals, from diaminotoluenes to benzocaine. Actually, natural henna has very low allergic potential, and allergic reactions are caused by the chemical colouring additives that are added to henna mixtures to improve or modify dyeing. Several products marketed as henna do not contain any natural constituent or only in low quantity.



picramic acid picramate benzocaine

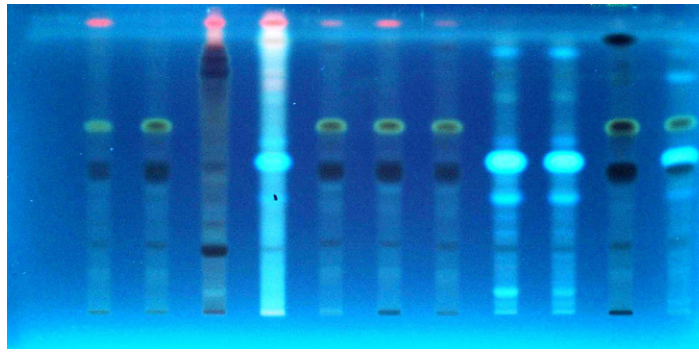


FIGURE 23 HPTLC fingerprint of henna market products. Mobile phase: ethyl acetate, formic acid, water (82:9:9). Derivatization: NPR and Macrogol. Visualization: UV366nm. Tracks: 1, lawsone (not visible in these conditions); 2,3,6,7,8, *Lawsonia inermis*; 4, *Cassia obovata*; 5, *Indigofera tinctoria*; 9, 10, *Isatis tinctoria*; 11, 12, products based on mix of Lawsonia with other plants: the track 11 evidences major content of *L. inermis* and track 12 appears like a mixture.

It is now clear the quantity of arguments calling for the intervention of HPTLC. In comparison with other techniques, HPTLC can give a set of analytical data able to provide a more complete figure of these very complex products, that we use to call henna. As a matter of fact, there is great concern about the quality determination of imported raw materials for producers and about the safety of dyeing products. It is conceivable that henna consumes will dramatically increase in the next years, owing to a request as cosmetic dye for grey hair in connection with the ageing necessities and as alternative to permanent tatoo.

HPTLC application on henna started in 2007 with determination of lawsone in henna powders, showing considerable variation from 0.004% up to 0.608%wt (Fig. 23) [24].

Later, the HPTLC fingerprint allowed to compare raw materials, powders collected from starting materials producers and their commercial products (Figs. 24 and 25).

The study was completed with the comparison of fingerprints of *L. inermis*, *I. tinctoria* and *C. obovata*, by three-dimensional visualization. HPTLC fingerprints were compared with those obtained by HPLC which gave similar results [24].

Coloured Investigations: The Jinshenkang Case

So far, limited but significant cases of adulteration, substitution or changes, with the aim of improving efficacy, have been reported and could invert the positive attitude of consumers about botanicals. Dangers mainly come from the utilization way. Registered products are prescription drugs that must be used under medical supervision, whereas herbal products are self administered and generally regarded as being harmless because of their natural origin. Quantity is

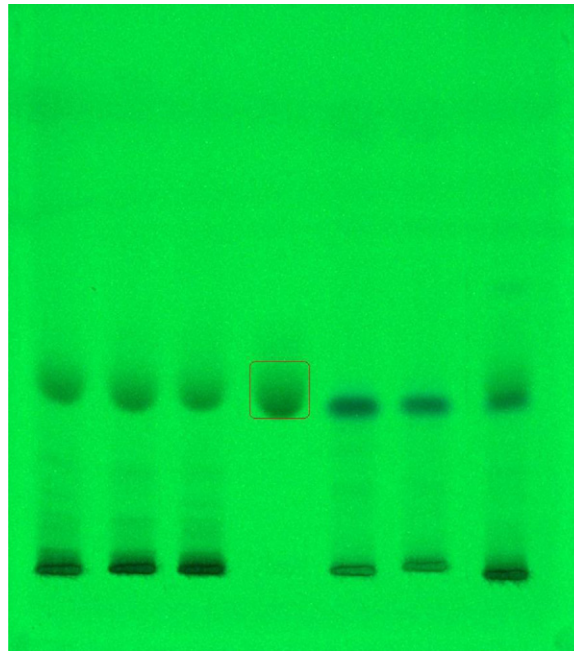


FIGURE 24 HPTLC of raw materials used for henna products in comparison with lawsone. Mobile phase: ethyl acetate, methanol, NH_4OH 5N (60:15:5). Visualization: UV254nm. Tracks: 1–3, *Lawsonia inermis*; 4, lawsone; 5, 6, *Isatis tinctoria*; 7, henna product containing both plants (sample of track 12 in Fig. 23).

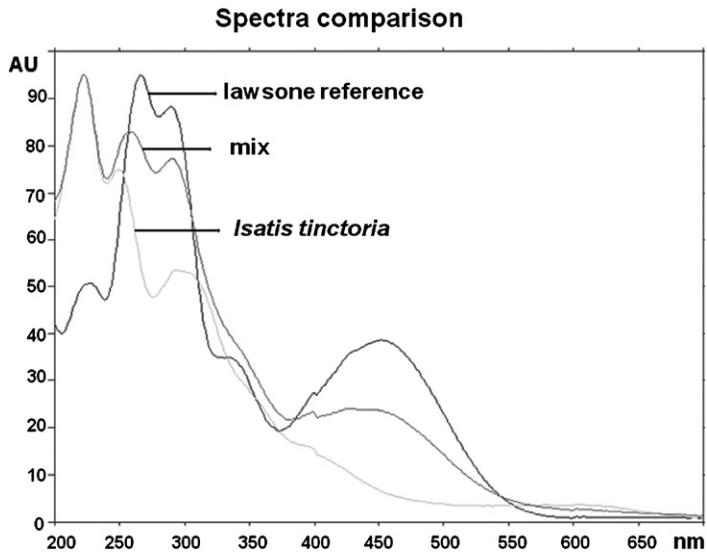


FIGURE 25 UV comparison of henna products with lawsone as reference confirmed the analysis of Fig. 24. Tracks: lawsone as reference, *Isatis tinctoria*, and product (track 7 in Fig. 24) containing a mix of *Lawsonia inermis* and *I. tinctoria*.

strictly considered in case of constituents of prescription drugs, whereas in food supplements the frequent and additional use is conceived and often promoted, as a consequence of the erroneous paradigm "natural herbs and plants are always safe". However, if natural substances can be dangerous, synthetic products are potentially toxic. Meanwhile, the phytovigilance alerts about important negative effects due to the spiking between natural and synthetic ingredients.

Controls should be based on simple, viable, comprehensible and low cost methods, but, in particular in this case, speed is a pre-requisite since deceitful producers use to change frequently composition and traceability, in order to avoid controls and punishments. In several cases an expert glance at the right HPTLC is enough to pick up the adulteration and go on with further studies.

As well known most problems come from the products that can be obtained by Internet-based sales, in particular an increasing market including herbal dietary supplements for the enhancement of sexual function and promoted as natural alternative to Viagra for curing impotence [25]. In these cases adulteration is a purposeful falsification. Emblematic the study that was performed on the product named Jinshenkang, or Sensual Tea, heavily present in Internet websites as able to rapidly solve any sexual problem of females and males without any collateral effect. The composition reported several herbal extracts, including Asiatic ginseng and other plants considered as aphrodisiacs. The examined product, once also marketed in Italy, came from Spain where first analyses did not evidence any adulteration (the product was now removed from the market). On the contrary, HPTLC showed a suspect great spot very strong at 366nm UV lamp (Fig. 26).

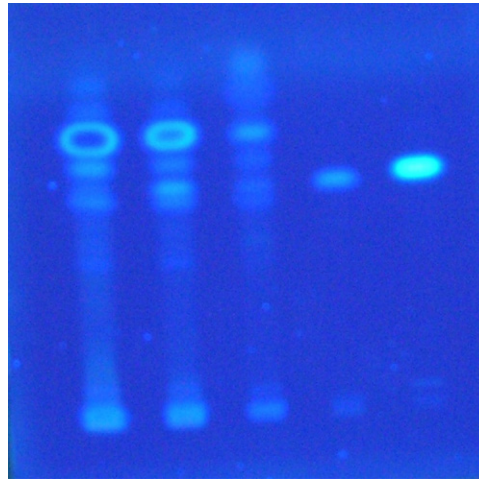
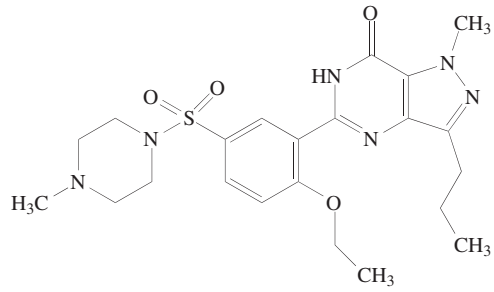
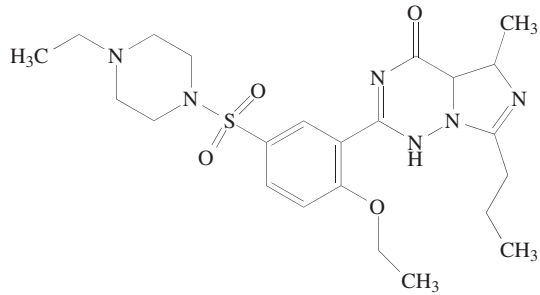


FIGURE 26 Comparison between Jinshenkang and PDE-5 inhibitors. Mobile phase: dichloromethane, methanol (9:1). Detection: UV366nm. Tracks: 1–3, ethyl acetate extracts of Jinshenkang at different concentrations; 4, sildenafil; 5, vardenafil.



sildenafil



vardenafil

Its Rf value was very similar to those of sildenafil and vardenafil, the PDE-5 inhibitors approved as anti-impotence drugs for the treatment of erectile dysfunction, but the sensitivity of HPTLC plate excluded the identity with these substances, as also confirmed by densitometry analysis, that showed different UV absorptions. A simple extraction with ethyl acetate afforded a complete removal of the substance from the product and the direct NMR spectrum of the dried extract resulted in an almost pure compound identified as thiosildenafil (Fig. 27).

The analysed Jinshenkang product came from 2009 market, whereas the analysis of a 2008 product revealed the unique presence of sildenafil: therefore, thiosildenafil, which has the same structure of sildenafil with an S instead of O, was artfully inserted to bypass the controls, too specific and specialised, as really happened. Both HPLC and HPTLC spectrometric analyses allowed ascertaining a quantity of the adulterant compound slightly superior to that contained in a tablet of Viagra. Thiosildenafil has been detected in another Chinese similar supplement, but using complicated 2D and 3D DOSY-COSY ^{1}H-NMR spectroscopy experiments [26], whereas other derivatives of sildenafil were detected using different analytic approaches [27–29].

Sildenafil, tadalafil and vardenafil, all PDE-5 inhibitors, have been approved as anti-impotence drugs for the treatment of erectile dysfunction. These are prescription drugs and should be only taken under the supervision of a physician,

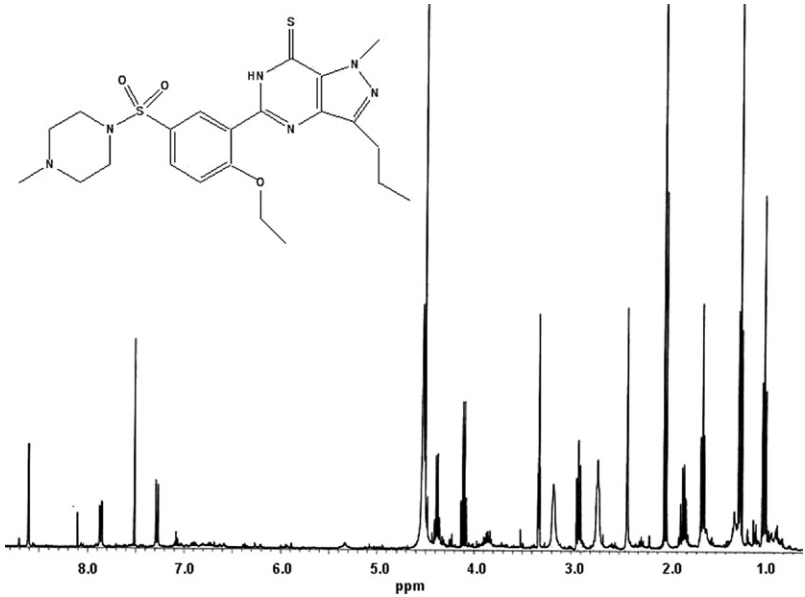


FIGURE 27 ^{1}H-NMR spectrum of the ethyl acetate extract of Jinshenkang (without any purification). The 10-*N*-methyl group in the pyrazolepyrimidine moiety changes its chemical shift (at ca. 4.5ppm), in comparison with the spectrum of sildenafil (at ca. 4.15ppm), due to the different interactions with the close heteroatom (S and O, respectively).

because of their side effects, such as headache, flush, dyspepsia, rhinitis, back pain and colour visual disturbances. Adulterated materials can therefore cause serious health problems if they are consumed as herbal "natural" products, generally considered deprived of toxicity by the consumers. It is evident that some manufactures act minor modifications on these structures to produce illegal products containing such analogues in order to circumvent controls. To our knowledge, more than eight of such adulterants have been reported, including the thio derivative.

Also adulteration is a job, a questionable but complicated work. Thus falsifiers know perfectly methods of investigation and adopt counteractions usually changing the composition of spiked products meanwhile or before control analyses are performed. To be effective analysis must be not only precise, reliable, up to date, but also flexible, clear and chiefly rapid.

Other Examples

Other easy evidences of adulteration were obtained for the presence of nimesulide and bromhexine in some Italian food supplements. In the first case, the label reported only plant extracts but a simple HPTLC silica gel plate in toluene:ethyl acetate 2:8 for the ethyl acetate extract of the product practically

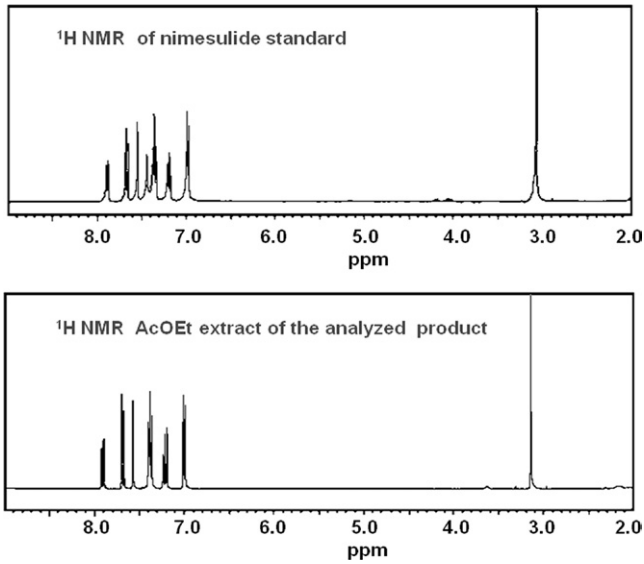
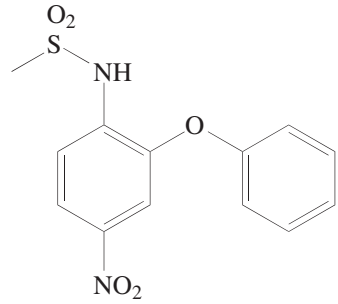


FIGURE 28 The confirmation of the nimesulide presence comes from the ^{1}H-NMR spectrum of the ethyl acetate extract of the market product.

showed a single main spot very evident in UV254nm light, with the same Rf of nimesulide standard. Often the presence of such strong spot is a good and immediate clue of the presence of synthetic ingredients. Direct NMR of the ethyl acetate extract totally confirmed the identification (Fig. 28).



nimesulide

The same analysis performed in another product of the same company containing several extracts, including that of *Adathoda vasica*, allowed to evidence the presence of bromhexine (Fig. 29). In this case adulteration is more intriguing, being bromhexine a hemisynthetic product obtained by transformation of the alkaloid vaccine, contained in the same plant.

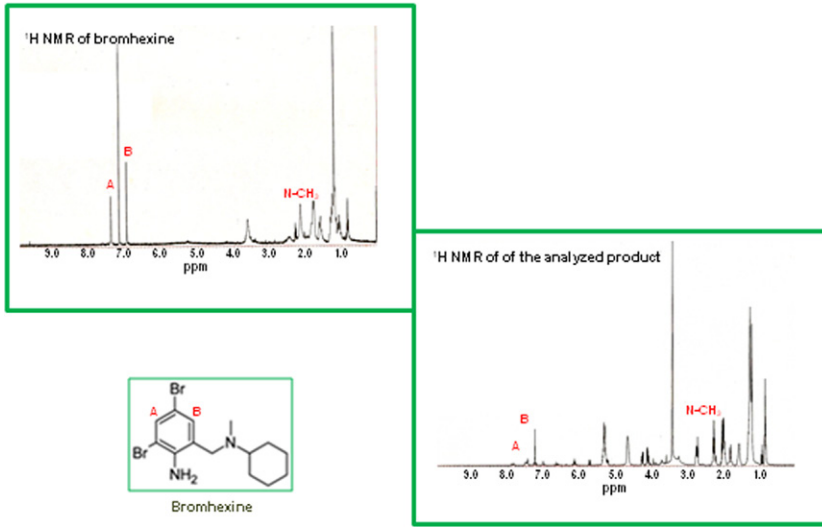


FIGURE 29 Marker peaks of bromhexine can be detected in the ^{1}H-NMR of the product ethyl acetate extract.

Finally, the presence of indole alkaloids of reserpine type was revealed by HPTLC in a product recommended for the sedative properties, where no plants containing alkaloids were reported in the composition.

In comparison with other techniques, HPTLC allows immediate evidence, useful for a quick intervention, and permits the easy differentiation between very similar substances, but the exact determination of novel substances needs further analyses, namely the identification by NMR or MS, without or after separation.

Experimenting

The current exploration/exploitation of plant natural substances for their transformation seems to be endless. New forms appear practically spontaneously and later wander looking for the necessary validation. The validation strictly depends by the available and selected scientific tools. In the case of products derived from extraction of the buds, generally obtained by the use of a ethanol/glycerine mix, the effort is just started. These products have so far a restricted market, being mainly present in France, Italy and Germany.

In consideration of some characteristics, including the dilution method, they are associated to the homoeopathy, as reported in the French Pharmacopoiea. [30]. Nowadays, this is the only legal recognition, where the method for the preparation of these products is also reported. However, not all the manufacturers follow these indications, increasing the confusion. Therefore, several products are present, such as M.G., i.e., glyceric macerate, or 1 DH, i.e., dilution according to the Homeopatic method, etc. (Fig. 30).

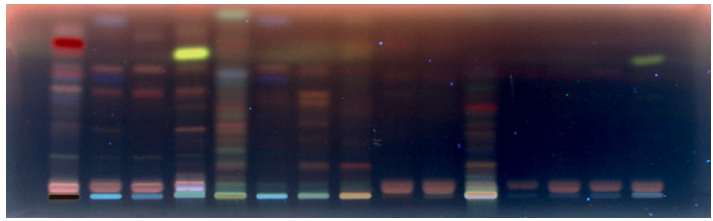


FIGURE 30 Preliminary analysis of gemmoderivatives. Mobile phase: dichloromethane, methanol (9:1). Derivatization: anisaldehyde. Visualization: UV366nm. Tracks: 1, *Ribes nigrum* MG; 2, Ficus MG; 3, Tilia MG; 4, Rosmarinus MG; 5, *Ribes nigrum* EI; 6, Ficus EI; 7, Tilia EI; 8, Rosmarinus EI; 9, *Ribes nigrum* 1DH; 10, *Ribes nigrum* MG; 11, *Ribes nigrum* EF; 12, Ficus 1 DH; 13, Tilia 1DH; 14, Tilia MG; 15, Rosmarinus 1 DH.

The relationship between homoeopathic products and the meristematic nature of the used plant organ generated the common idea that these products should contain irrelevant quantities of constituents and some primary metabolites accompanied by few enzymes. In any case the quantities of the constituents should be very low and therefore the sensibility of the method should be very high. Furthermore, the analyses are complicated by the relevant presence of glycerine. Another querelle concerns the real pharmacological properties of these products, generally considered like a placebo. However, if the activity is quite difficult to ascertain, the determination of the composition is another matter.

The HPTLC fingerprint approach was applied to this type of products in order to reach two main goals: to evidence the presence of constituents and to test their possible role in the identification of the utilized raw material.

The analysis of several products obtained from buds of several species evidenced tracks containing spots of several related secondary metabolites, in accordance with a specific fingerprint (Fig. 30). To obtain the fingerprint a concentration of the products was necessary.

The comparison with the mother tinctures showed a relevant correspondence, allowing an easy botanical identification. The main argument in favour of HPTLC was the possibility to limit the interference of glycerine, that was practically confined near the starting line with limited or null influence on the chromatogram.

The products obtained in accordance with the French Pharmacopoeia responded better to the separation, whereas products obtained using more polar solvents presented several problems that must be solved with solutions *ad hoc*.

Therefore, the study can be considered a first necessary step in the pathway for the validation of these products. On the basis of the HPTLC analyses performed, the following considerations can be proposed:

(a) HPTLC can be used to evidence the compositions of these products also in cases of complex mixtures

(b) the compositions of these products can be visualised and compared with those of ordinary extracts

(c) the fingerprints of different products of the same plant appear similar but not equal, confirming the differences due to the different processes
(d) the interference of glycerine can be limited owing to its maintenance near the deposition line.

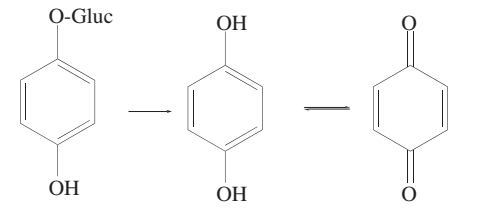
Due to the good results so far obtained further studies are now in progress to improve quality and visualization of the plates.

Limits and Shadows: The Bearberry Case

Nowadays, uses of herbal products are growing as well as the request of the global market for new useful species that causes also the introduction of alternative herbs instead of those traditionally used. HPTLC fingerprint was applied to evaluate the quality of marketed products based on bearberry leaf, commercially available as starting plant material (raw plant), in order to ascertain identity, and to detect eventual adulterations.

However, the analysis resulted more complicated than expected and therefore a multidisciplinary approach, also based on NMR and HPLC, was performed to identify *Arctostaphylos* spp. and the alternative plants, used in food supplements. In particular, the study revealed the presence besides the official species *Arctostaphylos uva-ursi* of other species and it was focused on *Arctostaphylos pungens* (=*A. glauca*), owing to its increasing use instead of the traditional bearberry.

A. pungens is easy to collect in good quantity in Mexico, whereas the European *A. uva-ursi* is even more difficult to find and costly. The first species is nowadays increasingly used instead of the second, a well-known medicinal plant traditionally and therapeutically employed to treat urinary infections [31–33]. In bearberry the activity is attributed to arbutin, a glucoside metabolically converted into the corresponding aglucone, hydroquinone and then paraquinone. *A. pungens* has morphological similarities with the official bearberry [34], but it presents a different story and can boast very few phytochemical data, including a high content in tannins (ca. 10%) and opposite information about the presence of arbutin.



Initially, HPTLC was used in order to obtain a clear chemical identification of the species present in the products (Fig. 31).

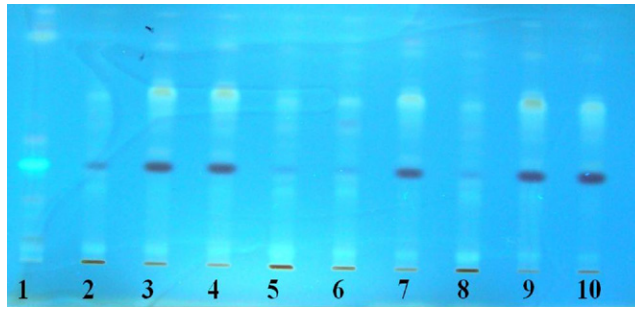


FIGURE 31 HPTLC of bearberry products. Mobile phase: ethyl acetate, formic acid, water (82:9:9). Derivatization: NPR and Macrogol. Visualization: UV366nm. Tracks: 1, unknown; 2, 5, 6, 8, *Arctostaphylos pungens*; 3, 4, 7, 9, 10, *A. uva-ursi*.

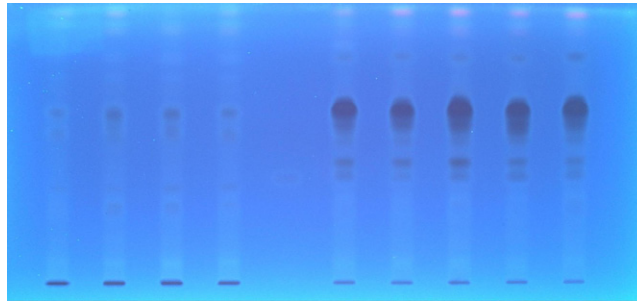


FIGURE 32 HPTLC of bearberry products. Mobile phase: ethyl acetate, formic acid, water (82:9:9). Derivatization: NPR and Macrogol. Visualization: UV366nm. Tracks: 1–4, products containing *A. pungens*; 5, arbutin; 6–10, products containing *A. uva-ursi*. The plate evidences the differences between the two groups, albeit the fingerprints are very similar. The zone corresponding to the arbutin is quite complicate in *A. pungens*.

The overall plate showed the presence of two groups of tracks: the one in accordance with *A. uva-ursi* and the other assignable to the *A. pungens* products (Fig. 32).

The assignments were confirmed by the presence of arbutin, as a clear strong spot in the bearberry's fingerprint, whereas in the *A. pungens* track the same zone appeared confused and difficult to understand (Figs. 31 and 32).

The ^{1}H-NMR spectrum of the ethyl acetate extract of the products of the two species gave results similar to the above ones, with analogous two sets of spectra, where the aromatic part showed clearly the pair of doublets due to the AA′BB′ system of arbutin in *A. uva-ursi* spectra, whereas in the *A. pungens* spectra the same zone was crowed of peaks, probably due to flavonoids, on the contrary practically absent in *A. uva-ursi* spectra (Fig. 33). Thus the pair of doublets typical of arbutin is not evident in *A. pungens*, being overlapped and confused with a great quantity of other peaks.

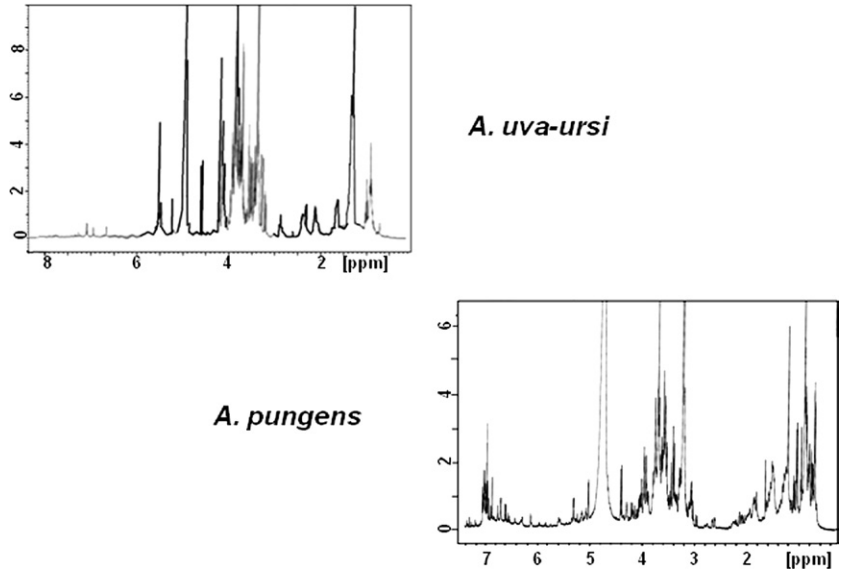


FIGURE 33 The ^{1}H-NMR spectrum of the ethyl acetate extract of the products of the two species. On the top a typical spectrum of *A. uva-ursi*; on the bottom, a typical spectrum of *A. pungens*. Attention must be focused to the signals at lower fields (left parts): in this part of the *A. uva-ursi* spectrum only the signals of the aromatic part of arbutin are evident, whereas in the pungens a quantity of peaks do not make possible any assignment.

However, the expert analyst knows some tricks to discover the clues of the analytic puzzle. The Gibbs reagent (2,6-dibromoquinone-4-chloroimide) is more sensitive to phenols, like arbutin, than NPR previously used. Using this revelation method further pushes were obtained to deepen the analysis, showing the presence of arbutin in *A. pungens*, also in low quantity.

Therefore, the identities of the utilised species were easily obtained, but not the arbutin content. This is an important point, since, as reported in European Pharmacopoeia [35], if the bearberry is used for therapeutic purposes the amount of arbutin must not be inferior to 7%, although so far this is not applied to food supplements. Quantitative determination can be afforded by the HPTLC densitogram (Fig. 34).

Again, the HPTLC method gave interesting results, but failed in affording an absolute quantitative determination on the content, because of overlapping of peaks, as evident also in UV analysis on the zone at the arbutin Rf (Fig. 35).

However, HPLC-MS investigation was able to separate the peaks of this area and identify unequivocally the presence and quantity of arbutin. The substance was present in very different amounts in the two groups of products: arbutin in *A. pungens* resulted in low amounts (0.5–1.3%), much lower than those of *A. uva-ursi*, as obtained by the HPLC-MS analysis according to the literature

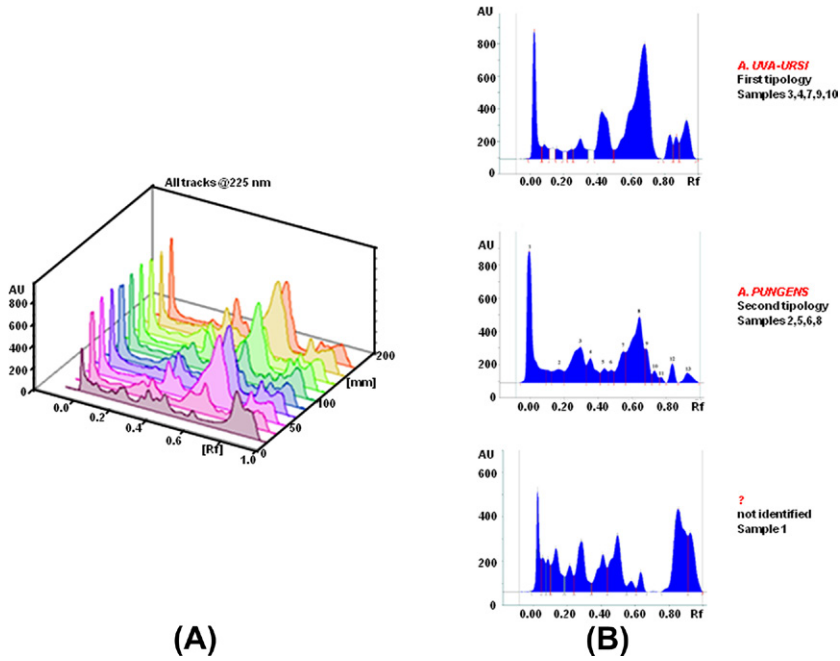


FIGURE 34 (A) 3D Densitometric analysis of samples named "Bearberry". (B) Their different fingerprints.

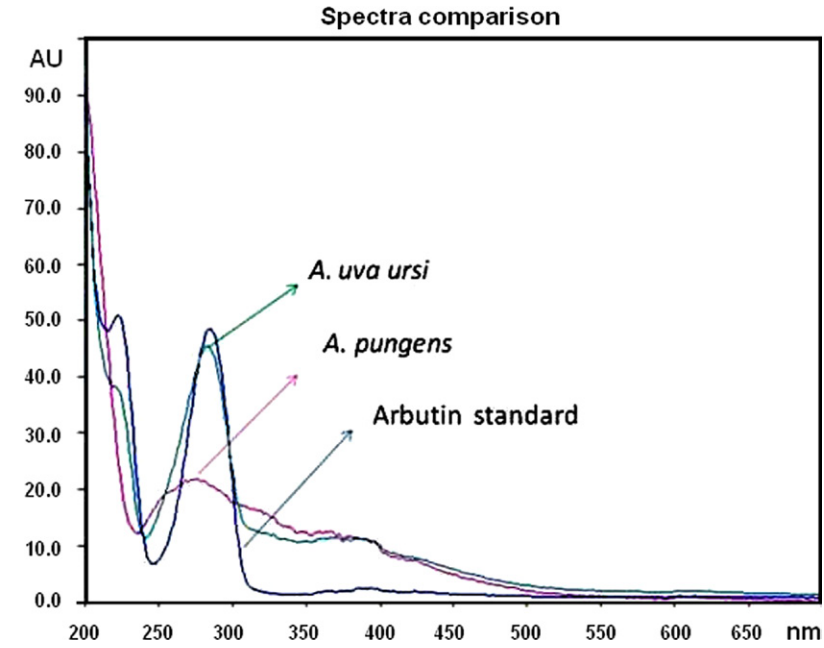


FIGURE 35 UV spectra on the spots at the same Rf of the arbutin standard in *A. uva-ursi* and *A. pungens* samples.

data (6.30–9.16%) [36]. Thereby, the replace of bearberry with *A. pungens* would lead to a decline in activity, but other considerations can be advanced.

The beneficial effects of bearberry are widely appreciated and its antimicrobial activity *in vitro* is reported in several studies. It is assumed a pre-drug mechanism, where arbutin is adsorbed from the gastrointestinal tract unchanged and then hydrolyzed in alkaline urine into hydroquinone, responsible of the antiseptic and astringent effects. Although bearberry is considered safe for oral use, hydroquinone, used at high doses as single substance, can cause nausea, vomiting, [37] shortness of breath, cyanosis, convulsions, delirium and collapse and are reported oxytocic (inducing or increasing the rapidity of labour) and chronic use, especially in children, can cause liver impairment from the metabolites. Therefore, on the light of reported negative effects of hydroquinones, lower quantities of arbutin in *A. pungens* could be positive, if compensated by the action of flavonoids, whose anti-bacterial and anti-inflammatory activity in urinary infections has been reported [38], albeit scientific validations on *A. pungens* activity is necessary to confirm this hypothesis.

CONCLUSIONS

So What

In the comparison with HPTLC fingerprint applications, HPLC wins for better resolution and sensitivity. However, the HPTLC method is rapid, universal, most cost-effective, easier and immediate in parallel comparison, identification and evident interpretation. Probably, the solution will be in a further improving of HPTLC resolution with sharper spots (experiments with nanoparticles are in progress) and in hyphenated techniques, like the already operating HPTLC/MS or the incoming HPTLC/NMR. Meanwhile, colourful impressive images of HPTLC fingerprints can be interpreted also by inexpert but interested observers, renovating the unique miracle of pulling down the chemical puzzles and revealing the micromolecular world. So far, a unique privilege of planar chromatography.

The Nowadays Future

The word “fingerprint” evokes the scenario of the individual molecular DNA identification. However, the use of Molecular Biology, of CSI’s type, is not possible for plant extracts [39] and very costly in the other useful cases. The aforementioned approach of the introduction of HPTLC fingerprints in Chinese Pharmacopoeia is important and clearly goes in the direction of the formation of a reference Data Bank for each species or each extract. The formation of such generated HPTLC data bank would be generally accepted when the identification, that means the comparison of tracks, will be obtained by computer analysis. Thus, a HPTLC plate, performed in the defined conditions, is converted in its high quality digital version and it is sent to the HPTLC Data Bank, where it

will be analysed by the Computer Centre, the identification is quickly achieved and the reply is obtained and sent. Similarly, information about the differences with the standard tracks can be reached. In this way, if the HPTLC analysis and its interpretation could be assigned to specialised centres, the cost of the determination of quality for botanicals will be greatly decreased.

The Day After

It is possible that planar chromatography will follow the pathway signed by the evolution of scientific thought, culminated in the multidimensional approach revealed by maths and physics. TLC raised into a Cartesian bi-dimensional world marked by Rf values. HPTLC introduced the third dimension with the determination of the quantity, that means contents of the substances. If the technology will give us the possibility to assign easily structures to the spots (as already started with hyphenated HPTLC) and will furnish fingerprints of metabolic markers in subsequent steps of a physiological status, the door to the metabolomic world will be open, at last. From the photographs to movies. The time, the forth dimension, will give us the possibility to follow live the devolution of a cancer cell or the developing of a pathology.

ACKNOWLEDGEMENTS

The authors wish to express their deep gratitude to Camag, Muttenz, Switzerland, that made possible the works reported in this paper by the invention of HPTLC powerful analytical devices.

REFERENCES

[1] M.S. Waltham, Roadmaps to Market: Commercializing Functional Foods and Nutraceuticals. Decision Resources, Inc., 1998, p. 5.
[2] Brookes, G., Economic Impact Assessment of the European Union (EU)'s Nutrition and Health Claims Regulation on the EU Food Supplement Sector and Market, for the European Health Claims Alliance (EHCA), (2010).
[3] Italian Medicines Agency (Agenzia Italiana del Farmaco-AIFA), The Use of Medicines in Italy-National Report-Year 2008. Pensiero Scientifico Editore, Rome, Italy, 2009.
[4] World Health Organization, Quality Control Methods for Medicinal Plant Materials. WHO Library Cataloguing, Geneva, 1998.
[5] M.S. Tswett, Berichte der Deutschen botanischen Gesellschaft. vol. 24, 1906, pp. 384–393.
[6] N. Pelick, H. Bolliger, H. Mangold, In: J.C. Giddings, R.A. Keller (Eds.), Advances in Chromatography, vol. 3, Marcel Dekker Inc., New York, 1966, pp. 85–118.
[7] R.E. Kaiser, J. Planar Chromatogr.-Mod. TLC 1 (1988) 182–199.
[8] E. Reich, A. Schibli, High-performance Thin-layer Chromatography for Analysis of Medicinal Plants. Thieme Medical Publishers Inc., New York, 2007.
[9] B. Renger, A. Benchmarking, J. Planar Chromatogr. 12 (1999) 58–62.
[10] E. Reich, In: J. Sherma, B. Fried (Eds.), Handbook of Thin-Layer Chromatography, third ed., Dekker, New York, 2003, pp. 135–157. Cap. 5.

[11] A. Piccin, M. Serafini, M. Nicoletti, Nat. Prod. Commun. 4 (2009) 1643–1644.
[12] J.-I. Zhang, M. Cui, Y. He, H.-I. Yu, De-An Guo, J. Pharm. Biomed. Anal. 36 (2005) 1029–1035.
[13] P. Krishnan, N.J. Kruger, A. Ratcliffe, J. Exp. Bot. 56 (2005) 255–265.
[14] C. Pereira, J.H. Yariwake, F.M. Lancas, J.N. Wauters, M. Tits, L. Angenot, Phytochem. Anal. 15 (2004) 241–248.
[15] The United States Pharmacopoeia, 31st ed., The National Formulary, 26th ed., The United States Pharmacopeia Convention, Rockville, 2000.
[16] Chinese Pharmacopoeia Commission, TLC Atlas of Chinese Crude Drugs in Pharmacopoeia of the People's Republic of China. People's Medical Publishing House, 2009.
[17] F.H.M. Do Monte, J.G. Dos Santos Jr., M. Russi, V.M. Lanziotti, L.K. Leal, G.M. Cunha, Pharmacol. Res. 49 (2004) 239–243.
[18] J.G. Dos Santos Jr., F.H.M. Do Monte, M.M. Blanco, V.M. Do Nascimento Bispo Lanziotti, M.F. Damasseno, L.K. De Almeida Leal, Pharmacol. Biochem. Behav. 81 (2005) 593–600.
[19] E.U. Graefe, M. Veit, Phytomedicine 6 (1999) 239–246.
[20] Council of Europe, European Pharmacopoeia, Supplement 6.1 to the sixth ed., EDQM, Strasbourg, 2008, pp. 3410–3411.
[21] A.J. Mordue, A. Blackwell, J. Insect Physiol. 39 (1993) 903–924.
[22] M. Nicoletti, M. Serafini, A. Aliboni, A. D'Andrea, S. Mariani, Parasitol. Res. 107 (2010) 89–94.
[23] The Scientific Committee on Cosmetic Products and Non-Food Products, Lawsone, Colipa n. C146, SCCNFP/0798/04, 2004.
[24] F.R. Gallo, G. Multari, M. Giambenedetti, E. Federici, Phytochem. Anal. 19 (2008) 550–559.
[25] L. Blok-Tip, B. Zomer, F. Bakker, K.D. Hartog, M. Hamzink, J. ten Hove, M. Vredenbregt, Food Addit. Contam. 21 (2004) 737–748.
[26] S. Balayssac, S. Trefi, V. Gilard, M. Malet-Martino, R. Martino, M.A. Delsuc, J. Pharm. Biomed. Anal. 50 (2009) 602–612.
[27] N. Daraghmeh, M. Al-Omari, A.A. Badwan, A.M.Y. Jaber, J. Pharm. Biomed. Anal. 25 (2001) 483–492.
[28] I. Wawer, M. Pisklak, Z. Chilmonczyk, J. Pharm. Biomed. Anal. 38 (2005) 865–870.
[29] H.J. Park, H.K. Jeong, M.I. Chang, M.H. IM, J.Y. Jeong, D.M. ChoiFood Addit. Contam. 24 (2007) 122–129.
[30] Ministere de Sanité Française, Xe éd., Pharmacopée Française, vol. 2. Agence Française de Sécurité Sanitarie de Produits de Sanité, 2007.
[31] British Herbal Pharmacopoeia. British Herbal Medicine Association, London, 1996.
[32] E. Yarnell, World J. Urol. 20 (2002) 285–293.
[33] D. Frohne, Planta Med. 18 (1970) 1–25.
[34] W. Applequist, The Identification of Medicinal Plants. A Handbook of the Morphology of Botanicals in Commerce. Missouri Botanical Garden, St Louis, 2006, pp. 27–28.
[35] Council of Europe, European Pharmacopoeia 6.0. vol. 2. EDQM, Strasbourg, 2008, p. 1794.
[36] I. Parejo, F. Viladomat, J. Bastida, C. Codine, Phytochem. Anal. 12 (2001) 336–339.
[37] J. Pizzorno, The Textbook of Natural Medicine. Churchill Livingstone, NewYork, 1999.
[38] H. Annuk, S. Hirmo, E. Türi, M. Mikelsaar, E. Arak, T. Wadström, FEMS Microbiol. Lett. 172 (1999) 41–45.
[39] P. Del Serrone, L. Attorri, B. Gallinella, F.R. Gallo, E. Federici, G. Palazzino, Nat. Prod. Commun. 1 (2006) 1137–1140.

Chapter 9

Natural Antioxidants (NAO) of Plants Acting as Scavengers of Free Radicals

Warjeet S. Laitonjam
Department of Chemistry, Manipur University, Imphal-795003, Manipur, India

INTRODUCTION

Over the past several years, there has been an extraordinarily rapid growth in our knowledge of free radical chemistry and its possible involvement in both normal essential biology and age related diseases and dysfunction. The oxygen molecule, which is essential for human beings and for many forms of life, is also responsible for the deterioration of organic molecules exposed to air.

Oxidative stress, defined as "the imbalance between oxidants and antioxidants in favour of oxidants, potentially leading to damage", is an important biochemical condition present in several human diseases and is most pronounced in autoimmune and inflammatory diseases such as ischemic stroke and rheumatoid arthritis [1–3]. In addition, the involvement of oxidative stress in cancer and neurodegenerative diseases such as familial amyotropic lateral sclerosis, Alzheimer's disease, Parkinson's disease, as well as diabetes mellitus, has recently been postulated.

Oxidative stress is characterized by the presence of unusually high concentrations of toxic reactive species, principally consisting of reactive oxygen species (ROS), reactive nitrogen species (RNS), reactive nitrogen oxygen species (RNOS), and unbound adventitious metal ions. Oxidative stress is believed to be a primary factor in various degenerative diseases of the central nervous system, as well as in the normal process of ageing both by inducing damage to mitochondrial DNA and by other mechanisms. The ROS formed during normal metabolic processes can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides thereby threatening the integrity of various biomolecules including proteins, lipids as well as lipoproteins in atherosclerosis and DNA – the so-called oxidative damage.

Bioactive Natural Products (Part Q), Vol. 37. http://dx.doi.org/10.1016/B978-0-444-59514-0.00009-2

OXIDATIVE DAMAGE DUE TO PHOTOCHEMICAL REACTION: FORMATION OF SINGLET OXYGEN ($^1O_2$)

Photochemically damaging events in cells are initiated by the uptake of the electronic energy of a photon by a UV-absorbing molecule. In the UV region of the electromagnetic spectrum, the energy of such photons is sufficient to break the covalent bonds. Usually, the absorbed energy converts the target molecule in its ground state to an electronically excited state, whose excess energy manifests itself in a different and often quite unstable electron configuration. The initial excited state, a short-lived singlet having fully paired electrons, may be deactivated by fluorescence (the emission of a photon having a longer than the exciting radiation) and return to the ground state; it may react with neighbouring molecules (although it is not common with singlets since their lifetimes are normally too short for them to diffuse over many molecular diameters); or it may undergo internal rearrangement to a longer-lived triplet excited state. The triplet state is more likely to react chemically with surrounding molecules [4,5].

A potential route for the formation of a damaging species from a photochemically activated triplet state is the transfer of triplet energy to molecular oxygen. The ground state of molecular oxygen has two unpaired electrons with parallel spin. Such a triplet state rare in ground state molecules and thus, because electrons occupying the same orbital must have opposed spin (the Pauli Exclusion Principle), the reaction of ground state oxygen with most substances is restricted. The activation of oxygen involves overcoming this spin restriction to reaction. Reduction leads to the potentially toxic superoxide anion, hydrogen peroxide and the hydroxyl radical. Electronic excitation of molecular oxygen, involving spin inversion, results in excited states with no unopposed spins, designated as singlet states.

Two excited singlet states of oxygen occur. The first singlet and the second singlet states are of 0.98eV and 1.63eV excitation energy, respectively. The second singlet state is extremely short-lived, being rapidly deactivated by collisonal quenching to the first singlet state, which has a life time long enough to allow chemical reaction. It is the first excited state of molecular oxygen that is involved in certain photooxidative, photodynamic and biological processes [6–8].

Although singlet oxygen ($^1O_2$) can be produced from various sources, including enzymes, the major mechanism of formation in biological systems is by energy transfer from photoexcited compounds or sensitizers. The absorption of a photon by such sensitizers, S results in an excited singlet state, ^1S with a very short life time (10^{-6}–10^{-8}s) (reaction (1)), which by intersystem crossing, involving spin inversion, may be relaxed to a longer-lived triplet state, ^3S (ca 10^{-3}s) (reaction (2)). Molecular oxygen, in a non-spin restricted reaction, can quench such a triplet state by energy transfer resulting in $^1O_2$ and the regeneration of the ground state sensitizer, S (reaction (3)).

$$S \rightarrow {}^1S \quad (1)$$

$${}^1S \rightarrow {}^3S \quad (2)$$

$${}^3S + {}^3O_2 \rightarrow {}^1S + {}^1O_2 \quad (3)$$

The relatively low excitation energy of its first excited singlet state allows oxygen to quench the triplet states of a variety of compounds. Several classes of biological molecules are susceptible to attack by $^1O_2$, including several protein amino acids – cysteine, methionine, tryptophan, and hystidine – with the possible consequences of enzyme inactivation. Membrane disruption, a common feature of photodynamic action, is due to lipid peroxidation initiated by the formation of hydroperoxides from the reaction of $^1O_2$ with unsaturated fatty acids. Of the nucleic acids, guanine is particularly sensitive to attack by $^1O_2$. Other classes of compounds may quench as well as react with $^1O_2$, example being the phenol α-tocopherol (α-TOH, Vitamin E) and certain amines. The most efficient naturally occurring physical quenchers of $^1O_2$ are the carotenoids [9–11].

OXIDATIVE DAMAGE DUE TO NON-PHOTOCHEMICAL REACTION: FORMATION OF FREE RADICALS AND ROS

Non-photochemical routes for oxidative damage in plants usually involve the interaction of molecular oxygen with free radicals to produce new, potentially harmful free radical species containing oxygen or ROS [12,13]. This type of reaction may occur directly, or it may be promoted by enzyme catalysts normally present in the plant cell, such as the enzyme lipoxygenase.

Atmospheric oxygen is unusual in that its ground state has two unpaired electrons; it is a triplet state with considerable di-radical character. This permits it to enter into energetically favourable chain reactions with many organic free radicals. The formation of organic (usually carbon-centred) free radicals $R^{\cdot}$ from non-radical precursors is called the initiation phase of the autooxidation. This process, which is often quite slow, results in the characteristic 'lag period' of a radical chain reaction. In the propagation phase of the reaction, there is build up of peroxy radicals, $ROO^{\cdot}$, and the subsequent reaction of peroxy radicals with compounds (R′H) having extractible hydrogen atoms. The new radicals, R′· are then available for further reaction with molecular oxygen. Finally, when all the oxygen or active hydrogen species are used up, the termination phase begins. In this phase, the radicals recombine with each other to produce inactive, non-radical products:

$$RH \rightarrow R^{\cdot} \qquad \text{Initiation}$$

$$R^{\cdot} + O_2(O^{\cdot} - O^{\cdot}, {}^3O_2) \rightarrow ROO^{\cdot} \qquad \text{Propagation}$$

$$ROO^{\cdot} + R'H \rightarrow ROOH + R' \qquad \text{Propagation}$$

$$ROO^{\cdot} + R^{\cdot} \rightarrow ROOR \quad \text{Termination}$$

$$2ROO^{\cdot} \rightarrow ROOR + O_2 \quad \text{Termination}$$

Hydrogen-donating antioxidants can react with lipid peroxyl radicals in a termination reaction, which breaks the cycle of generation of new radicals:

$$ROO^{\cdot} + AH \rightarrow ROOH + A^{\cdot}$$

where A represents an antioxidant. In the case of vitamin E, the resulting radical has a much longer half life than the lipid peroxy radical and so is much more stable. This, therefore, increases the chance of occurrence of a true termination reaction. In the case of flavonoids, the hydroxyl groups are the most important for hydrogen donation. In the case of the flavonols, hydroxyl groups at the 2', 3', and 4' positions increase the stability of radicals.

ANTIOXIDANTS

The term "antioxidant" is almost impossible to define, except for the chemical definition of an agent that prevents oxidation. Many attempts have been made, but the most useful definition is probably – "any substance, that when present at low concentration compared to that of an oxidisable substrate, significantly delays or inhibits oxidation of that substance [14]." The term antioxidant is a much used (and abused) term, and it is important to understand that an effective antioxidant in one assay system is not necessarily an effective antioxidant in another. For this reason, there is no single assay which can possibly define the total efficacy of an antioxidant. In assessing an antioxidant, it is important to concentrate on the mechanism of action, since there are many ways to generate free radicals, many ways in which a free radical can be quenched by an antioxidant, many factors which can influence this reaction, and an almost infinite number of possible targets of the free radicals. Antioxidant action arises from a combination of several distinct chemical events, which include: metal chelation; hydrogen donation from phenolic groups; partition coefficients; oxidation to a non-propagating radical; redox potential and enzyme inhibition. Furthermore, the health implications of a putative antioxidant depend upon how well it is absorbed by the body and how it is metabolized.

ROS AND FREE RADICALS

Before coming to the various natural antioxidants (NAO) of plants, I will briefly introduce some of the ROS and free radicals. ROS include a number of chemically reactive molecules derived from oxygen [15]. Some of these molecules are extremely reactive, such as the hydroxyl radical, while some are less reactive (superoxide and hydrogen peroxide). Intracellular free radicals, i.e., free low molecular weight molecules with an unpaired electron, are often ROS and vice

TABLE 1 The Major ROS Molecules and Their Metabolism

ROS molecule	Main sources	Enzymatic defence system
Superoxide ($O_2^{\cdot -}$)	"Leakage" of electrons from the electron transport chain	Superoxide dismutase (SOD)
	Activated phagocytes	Superoxide reductase (in some bacteria)
	Xanthine oxidase	
	Flavoenzymes	
Hydrogen peroxide (H_2O_2)	From $O_2^{\cdot -}$ *via* superoxide dismutase (SOD)	Glutathione peroxidase
	NADPH-oxidase (neutrophils)	Catalases
	Glucose oxidase	Glutathione peroxidase
	Xanthine oxidase	
Hydroxyl Radical $^{\cdot}$(OH)	From $O_2^{\cdot -}$ and H_2O_2 *via* transition metals (Fe or Cu)	Glutathione
Nitric oxide (NO)	Nitric oxide synthases	Peroxiredoxins

versa; and the two terms are therefore commonly used as equivalents. Free radicals and ROS can readily react with most biomolecules, starting a chain reaction of free radical formation. In order to stop this chain reaction, a newly formed radical must either react with another free radical, eliminating the unpaired electrons, or react with a free radical scavenger – a chain-breaking or primary antioxidant.

In Table 1, the most common intracellular forms of ROS are listed together with the relevant enzymatic antioxidant systems scavenging these ROS molecules.

Superoxide ($O_2^{\cdot -}$)

The superoxide anion radical created from molecular oxygen by the addition of an electron is, in spite of being a free radical, not highly reactive. It lacks the ability to penetrate lipid membranes and is, therefore, enclosed in the compartment where it was produced. The formation of superoxide takes place spontaneously, especially in the electron rich aerobic environment in vicinity of the inner mitochondrial membrane with the respiratory chain (Fig. 1). Superoxide (as well as hydrogen peroxide) is also produced endogeneously by flavoenzymes, e.g., xanthine oxidase. Other superoxide-production enzymes are lepoxygenase and cyclooxygenase. The NADPH-dependent oxidase of phagocytic cells,

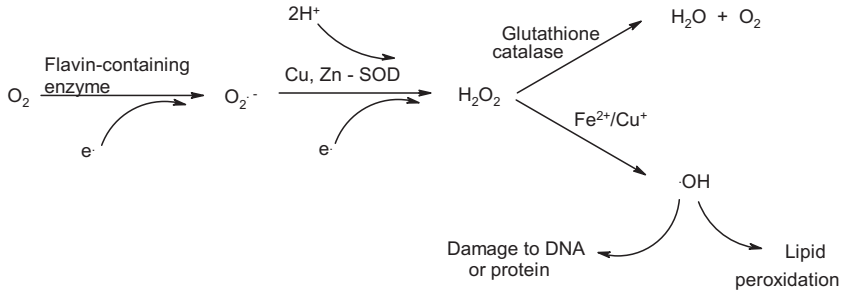


FIGURE 1 Simplified scheme for the ROS formation and metabolism on the intracellular level.

a membrane-associated enzyme complex, constitutes an example of deliberate high-level $O_2^{\cdot -}$ production. Enzymes similar to components of this complex are also present in non-phagocytic cells where their functions should be connected to signalling. Two molecules of superoxide rapidly dismutase to hydrogen peroxide and molecular oxygen, and this reaction is further accelerated by superoxide dismutase (SOD).

Hydrogen Peroxide (H_2O_2)

Hydrogen peroxide is not a free radical but is nonetheless highly important much because of its ability to penetrate biological membranes. It plays a radical forming role as an intermediate in the production of more reactive ROS molecules including hypochlorous acid (HOCl), by the action of myeloperoxidase, an enzyme present in the phagosomes of neutrophils and, most importantly, formation of hydroxyl radical ($^{\cdot}OH$) in oxidation of transition metals.

$$H^+ + Cl^- + H_2O_2 \rightarrow HOCl + H_2O$$

Another important function of H_2O_2 is carried out in its role as intracellular signalling molecule.

H_2O_2 once produced by the above-mentioned mechanisms is removed by at least three antioxidant enzyme systems – catalases, glutathione peroxidases, and peroxiredoxins.

Hydroxyl Radical ($^{\cdot}OH$)

Due to its strong reactivity with biomolecules, hydroxyl radical is probably capable of doing more damage to biological systems than any other ROS. The radical is formed from hydrogen peroxide in a reaction catalyzed by metal ions (Fe^{2+} or Cu^{+}), often bound in complex with different proteins or other molecules. This is known as the Fenton reaction:

$$H_2O_2 + Cu^+/Fe^{2+} \rightarrow {}^{\cdot}OH + OH^- + Cu^{2+}/Fe^{3+}$$

Superoxide also plays an important role in connection with the above reaction by recycling the metal ions:

$$Cu^{2+}/Fe^{3+} + O^{\cdot}_{2}{}^{-} \rightarrow Cu^{+}/Fe^{2+} + O_2$$

The sum of these two reactions is the Haber–Weiss reaction: transition metals thus play an important role in the formation of hydroxyl radicals. Transition metals may be released from proteins such as ferritin and the [4 Fe–4 S] centre of different dehydrases by reaction with superoxide. This mechanism, specific for living cells, has been called the *in vivo* Haber–Weiss reaction.

Nitric Oxide (NO)

Nitric oxide represents an odd member of the free radical family and is similar to superoxide in several aspects in that it does not readily react with most biomolecules despite its unpaired electron. On the other hand, it easily reacts with other free radicals (e.g., peroxyl and alkyl radicals), generating mainly less reactive molecules, thus in fact functioning as a free-radical scavenger; NO has, for example, been shown to inhibit lipid peroxidation in cell membranes. Though, if superoxide is produced in large amounts in parallel with NO, the two react with each other to give $OONO^-$ (peroxynitrite), which is highly cytotoxic. Peroxynitrite may react directly with diverse biomolecules in one- or two-electron reactions, readily react with carbon dioxide to form highly reactive nitroso peroxocarboxylate ($ONOOCO_2^-$), or protonated as peroxonitrous acid (ONOOH) undergo homolysis to form either $^{\cdot}OH$ and $^{\cdot}NO_2$ or rearrange to nitrate (NO_3). The individual rates of these different reactions of peroxynitrite will depend upon the pH, temperature, and type of compounds present in the surrounding milieu. Peroxynitrite, directly or *via* its reaction products, may oxidize low-density lipoprotein (LDL), release copper ions by destroying ceruloplasmin, and generally attack tyrosine residues in different proteins, as observed in many inflammatory diseases.

ROS are, due to their high reactivity, prone to cause damage, and are thereby also potentially toxic, mutagenic, or carcinogenic. The targets for ROS damage include all major groups of biomolecules, such as DNA, lipids and proteins.

CELLULAR ANTIOXIDANT ENZYME SYSTEMS

Antioxidant activity is essential for life, to counter act the strongly oxidizing environment in which we live. Many biological functions, such as protection from mutagenesis, carcinogenesis, and ageing, among others, are due to antioxidative effects. The antioxidant activities of several plant materials have recently been described. There has been increasing interest in oxygen-containing free radicals or ROS in biological systems and their implied role as causative agents in the aetiology of a variety of chronic disorders. Accordingly,

attention is being focused on the protective biochemical functions of naturally occurring antioxidants in the cells of the organisms containing them, and on the mechanism of their action. Some of the antioxidant enzyme systems serving to protect cells and organisms from the lethal effects of excessive ROS formation are:

(i) *SOD*: SOD was the first genuine ROS-metabolizing enzyme discovered. SOD catalyzes the conversion of superoxide (O_2^-) to hydrogen peroxide and oxygen. In the reaction catalyzed by SOD, two molecules of superoxide form H_2O_2 and molecular oxygen, and are thereby a source of cellular hydrogen peroxide.

$$2O^{\cdot}{}_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

In mitochondria, superoxide is formed in relatively high concentrations due to the leakage of electrons from the respiratory chain.

Both SOD and catalase activity have been shown to decline in the older leaves of tobacco plants, which revealed signs of membrane damage. There were clear corrections between the activity of these two enzymes and the degree of lipid peroxidation in the leaves. Both enzymes were important agents for protecting leaves from the deleterious effects of membrane lipid destruction [16].

(ii) *Superoxide reductases (SOR)*: recently, a novel type of superoxide scavenging enzyme was discovered, catalyzing the direct reduction of superoxide:

$$O^{\cdot}{}_2^- + e^- + 2H^+ \rightarrow H_2O_2$$

These enzymes, the SOR, contain iron and have so far only been found in anaerobic sulphate-reducing bacteria, here called desulphoferrodixin [17], the anaerobic archaea *Pyrococcus furiosus* [18] or the microaerophilic bacterium causing venereal syphilis, *Treponema pallidum* [19]. The latter organism lacks SOD and seems to rely solely on SOR for its elimination of superoxide. Yet there is no evidence for a mammalian SOR.

(iii) *Catalases*: catalases of many organisms are mainly haeme-containing enzymes. The predominant subcellular localization in mammalian cells is in peroxisomes, where catalase catalyzes the dismutation of hydrogen peroxide to water and molecular oxygen:

$$2\ H_2O_2 \rightarrow O_2 + 2\ H_2O$$

Catalase also has functions in detoxifying different substrates, e.g., phenols and alcohols, *via* coupled reduction of hydrogen peroxide:

$$H_2O_2 + R'H_2 \rightarrow R' + 2H_2O$$

One antioxidative role of catalase is to lower the risk of hydroxyl radical ($^{\cdot}OH$) formation from hydrogen peroxide *via* the Fenton reaction catalyzed by Cu or Fe ions [15]. Catalase binds NADPH, which protects the enzyme from inactivation and increases its efficiency.

(iv) *Peroxiredoxins*: peroxiredoxins (thioredoxin peroxidases) are recently discovered enzymes capable of directly reducing peroxides, e.g., hydrogen peroxide and different alkyl hyderoxides [20]. In the mitochondria of mammalian cells, the mitochondrial thioredoxin system is probably a specific reductant of peroxiredoxins.

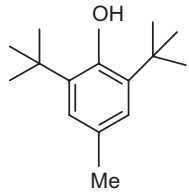
(v) *Glutathione peroxidases*: a tripeptide bearing a thiol group, glutathione (GSH) is found in very high concentrations in many cells. It reacts with many oxidants, such as H_2O_2 and other peroxides (e.g., lipid peroxides in cell membrane), to form the oxidized form, a disulphide known as glutathione disulphide, GSSG:

$$ROOH + 2GSH \rightarrow ROH + GSSG + H_2O$$

The above reaction is catalyzed in mammalian cells by an important selenium-containing enzyme, glutathione peroxidase. Some data have indicated that glutathione peroxidases should be of high antioxidant importance under physiological conditions, while others place the enzymes as important only at events of oxidative stress.

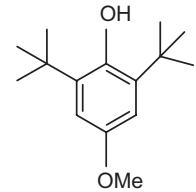
NATURAL ANTIOXIDANTS (NAO) OF PLANTS

Synthetic antioxidants, such as butylated hydroxytoluene (BHT, **1**) and butylated hydroxyanisole (BHA, **2**), have restricted use in foods and synthetic antioxidants are suspected to be carcinogenic [21]. Therefore, the search for NAO, especially of plant origin, has greatly been increased in recent years.



2,6-di-*tert*-butyl-4-methyl phenol, BHT

1



2,6-di-tert-butyl-4-methoxy phenol, BHA

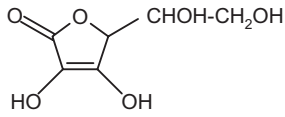
2

In recent years, researchers on antioxidant activities of medicinal plants have remarkably augmented by virtue of increased interest in their potential high antioxidant capacity and positive health benefits [22–24]. Compounds which are widely used as NAO are:

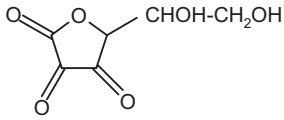
(a) *Ascorbic acid (Vitamin C), **3***: ascorbic acid (Vitamin C), **3** has been proposed for a long time as a biological antioxidant. It exists in rather high concentrations in many cellular environments, such as the stroma of chloroplasts. Ascorbate is water soluble and has been shown to be a major antioxidant in human plasma as well as in and even across cell membranes. It reduces α-*TOH* (Vitamin E), **5** as well as peroxides and ROS, such as

superoxide. The vitamin serves mainly to prevent lipid hydroperoxide formation in plasma lipoproteins, e.g., LDL, by reducing α-*TOH* (**5a**) radicals formed upon reaction with lipid peroxy radicals. This is, in turn, an important function in the prevention of atherosclerotic plaque formation. Ascorbate also protects lipids in cell membranes by this mechanism. Intracellularly, in the aqueous phase, ascorbate and glutathione act in concert to protect the cell from oxidative damage.

Ascorbate inhibited the photoxidation of a kaempferol by illuminated spinach chloroplasts. It reduces two equivalents of superoxide to produce hydrogen peroxide and the derivative *dehydroascorbic acid*, **4**. It also reacts with singlet oxygen at a relatively fast rate.



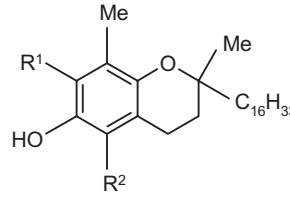
ASCORBIC ACID, **3**



DEHYDROASCORBIC ACID, **4**

(b) *Vitamin E, 5*: naturally occurring compounds with Vitamin E activity are the *tocopherols*, a group of closely related phenolic benzochroman derivatives having extensive ring alkylation. These compounds occur not only in plants but also in mammalian tissues. Like other phenolic antioxidants, e.g., **BHT**(**1**) and **BHA**(**2**), their normal mechanism of action is the inactivation of two equivalents of chain-carrying peroxy radicals, terminating two potential radical chain reactions per molecule of inhibitor. At lower radical concentrations, however, the potential exists for the regeneration of **Vitamin E**(**5**) through reaction of a reducing agent such as **Vitamin C**(**3**).

The most biologically active of the four major tocopherols is α-*TOH*, **5a**. α-TOH is a lipid-soluble vitamin present in biological membranes. It contains a hydroxyl group by which it reacts with unpaired electrons and can reduce, e.g., peroxyl radicals. α-TOH is absorbed in the intestine and transported to the liver in chylomicrons. It is returned to the blood in very low-density lipoprotein (VLDL) particles that with lipoprotein lipase are degraded to LDL. LDL particles provide cells in peripheral tissues with cholesterol and are also important as a factor in atherogenesis. Only 5–10 molecules of α-TOH exist in each LDL, in contrast to the abundance of oxidizable lipid molecules. This fact points out the need for compounds that can efficiently recycle α-TOH. In fact, since ascorbate recycles α-TOH and mammalian thioredoxin reductase reduces dehydroascorbate. Thioredoxin reductase may play an important role for the total *Vitamin E* antioxidant function. It has long been known that Se and *Vitamin E* have mutual sparing effects, and thioredoxin reductase could be the link explaining this fact.



5a, $R^1 = R^2 = Me$; α-Tocopherol
5b, $R^1 = H$, $R^2 = Me$; β-Tocopherol
5c, $R^1 = Me$, $R^2 = H$; Γ-Tocopherol
5d, $R^1 = R^2 = H$; δ-Tocopherol

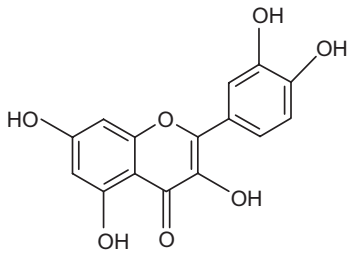
(c) *Flavonoids*: flavonoids are diphenyl propanes that commonly occur in plants (more than 4000 flavonoids have been isolated) and are frequently compounds of human diet. The immediate family members of flavonoids include flavones, isoflavones, and the 2,3-dihydroderivatives of flavones, namely, flavanones which are inter-convertible with the isomeric chalcones.

Epidemiological evidence suggests an inverse relationship between dietary intake of flavonoids and the risk of coronary heart disease. It has been suggested that oxidative modification of LDL plays an important role in the development of human atherosclerosis. Thus protecting LDL from oxidation by compounds such as flavonoids, may be an effective strategy to delay or prevent the progression of the disease.

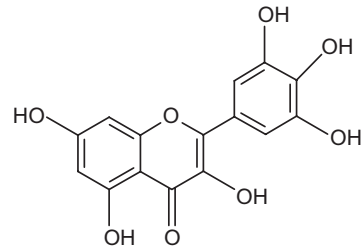
The biological activities of flavonoids have been extensively reviewed. Some of them have been found to possess anti-ischemic, anti-platelet, anti-inflammatory, anti-lipoperoxidant activities. Flavonoids have also been found to inhibit a wide range of enzymes involved in oxidation systems such as 5-lipoxygenase, cyclooxygenase, monooxygenase or xanthine oxidase. These biological activities are related to their antioxidative effects. Flavonoids can perform their antioxidant activity by various mechanisms, e.g., by scavenging radicals, by binding metal ions, and by inhibiting enzymatic systems responsible for free radical generation. In contrast to the beneficial effects, some flavonoids (such as *quercetin*, **6**) have also been reported to be mutagenic, and co-carcinogenic. These detrimental effects could be attributable to the peroxidant activity of flavonoids (generally linked to catechol or pyrogallol pattern) generating free radicals under certain conditions, e.g., in the presence of metal ions.

Flavonoids occur widely in the plant kingdom, and are especially common in leaves, flowering tissues, and pollens. They are also abundant in woody parts such as stems and barks. Several classes of flavonoids show antioxidant activity towards a variety of easily oxidizable compounds. The antioxidant efficiency of a group of flavonoids was measured in a test system incorporating two easily oxidized lipids – linolenic acid and β-carotene. It was found that the highest

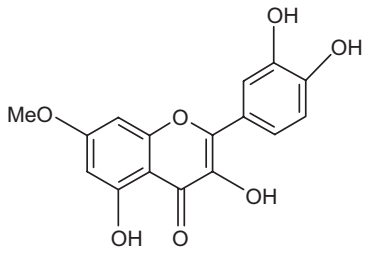
activities were shown by free flavonols – *myricetin*, **7** and *robinetin* – having three hydroxyl groups in the B-ring with a 3',4',5'-substitution pattern. Slightly inferior, but still significant activities were displaced by the pentahydroxy aglycones *quercetin*, **6** and dihydroquercetin; the 3-rhamnoside of quercetin; the tetrahydroxymethoxy flavonol *rhamnetin*, **8**; and the tetrahydroxy flavonol *fisetin*, **9**. Dihydroquercetin was isolated from Spanish peanuts (*Arachis hypogea*) and also shown to have high antioxidant activity in a tlc spray test using β-carotene.



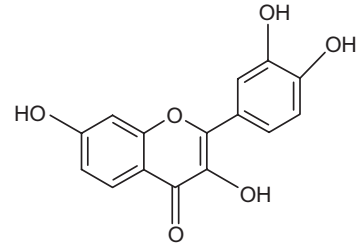
QUERCETIN, **6**



MYRICETIN, **7**



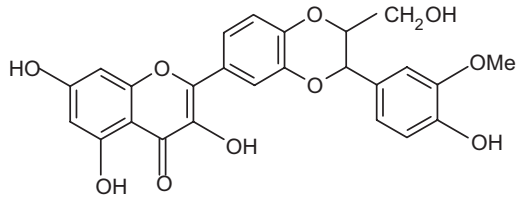
RHAMNETIN, **8**



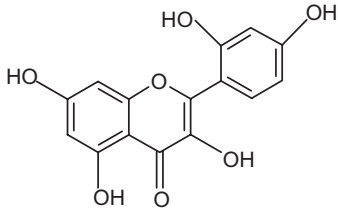
FISETIN, **9**

It was shown that *quercetin*, **6** and the complex flavonol-lignan derivative *silymarin*, **10** were about equally effective in inhibiting lipid peroxidation in several different michrosomal or mitochondrial preparations. When a series of flavonoids was tested as inhibitors for the autooxidation of emulsified linolenic acid and methyl linoleate in the dark at room temperature, it was found that the two compounds with highest activity were *morin*, **11** and *kaempferol*, **12**. They attributed the activity of flavonoids to their ability to donate a hydrogen atom to the peroxy radical derived from the autooxidizing fatty acid derivative. *Kaempferol*, **12** was also reported to undergo photobleaching in illuminated chloroplasts. The bleaching response was stimulated by methyl viologen (paraquat), a well-known electron transfer agent capable of producing superoxide from molecular oxygen, and it was suppressed

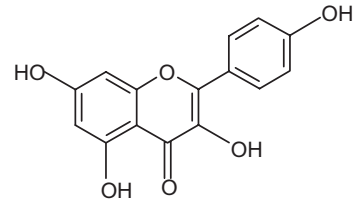
by SOD. These results suggest that flavonoids inhibit superoxide-promoted redox reactions within the chloroplast. Further evidence for this postulate was obtained in experiments that showed *quercetin*, **6** and *kaempferol*, **12** at about 4×10^{-5}M, suppressed lipid photoperoxidation in isolated spinach chloroplasts by 50%.



SILYMARIN, **10**



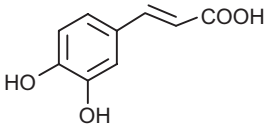
MORIN, **11**



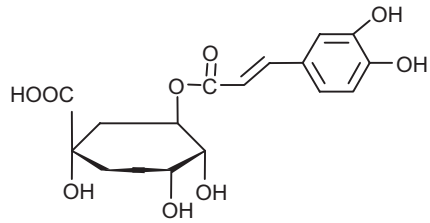
KAEMPFEROL, **12**

Several flavonoids were shown to be potent inhibitors of the enzymes lipoxygenase and prostaglandin synthetase, which convert polyunsaturated fatty acids to oxygen-containing derivatives. Highest activity against both enzymes was shown by *luteslin* (5,7,3',4'-tetrahydroxyflavone) and 3',4'-dihydroxyflavone. Many reports on the research of the antioxidant activity of flavonoids are found in the literature.

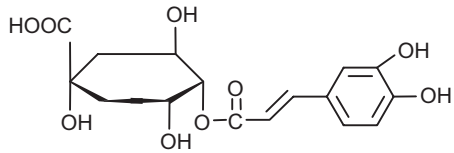
(d) *Phenolic acids*: acidic compounds incorporating phenolic groups have been implicated as active antioxidants. *Caffeic acid* (**13**), *Chlorogenic acid* (**14**) and its isomers, including *4-O-caffeoyl quinic acid* (**15**) were isolated from sweet potatoes. *Chlorogenic acid*, **14** was found to be the most abundant phenolic acid in the plant extract and also the most active antioxidant. Esters of caffeic acid with sterols and triterpene alcohols have been isolated from the seed of the grass *Phalanis canariensis*. The fatty acids of the seed were predominantly unsaturated, suggesting that the esters were acting to protect them from oxidation. The lipid-soluble esters were effective antioxidants in tests with lard or sardineoil heated at 60°C. In the tests, the esters were added as mixtures, but at least some components appeared to have activity approaching or exceeding that of BHT.



CAFFEIC ACID, 13



CHLOROGENIC ACID, 14



4-O-CAFFEOYLQUINIC ACID, 15

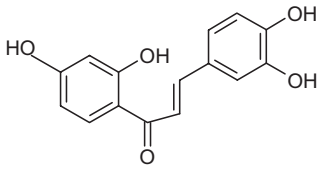
Ferulic acid was shown to delay the photooxidation of linoleic acid and esters of *freulic acid* with triterpenes and sterols, which occur naturally in the rice bran, were also shown to have some activity.

(e) Other phenols: *Rosmaridiphenol*, a diterpene derivative with adjacent OH groups, was isolated from *Rosmarinus officinalis* (rosemary). Its antioxidant activity in heated lard exceeded that of *BHA* and approached that of *BHT*. Related phenolic diterpenes with antioxidant activity have also been isolated from this plant.

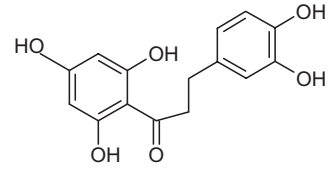
A group of potent antioxidants for the air oxidation of *linoleic* acid was isolated from the methanol extract of the rhizome of *Curcuma longa* (turmeric). The most abundant and most active constituent of the extract was the orange pigment, *curcumin*. The mechanism of *curcumin* activity may include metal ion chelation by the central β-diketone group. The *lignan A*, isolated from sesame (*Sesanum indicum*) seed, significantly inhibited the autooxidation of linoleic acid at 40°C. It was not as active as sesamol or vitamin E. Polyhydroxylated chalcones such as *butein*, which were biosynthetic intermediates between cinnamic acids and flavonoids, also show considerable antioxidant activity for lard. Hydrogenation of the chalcone double bond increased their antioxidant activity to some extent; e.g., the *pentahydroxydihydrochalcone* was ca. 2–3 times as active as the corresponding unsaturated chalcone.

Ubiquinol, a reduction product of ubiquinone (coenzyme Q), was shown to be a potent *in vivo* antioxidant under conditions of low oxygen concentration, such as would occur in many cellular environments. The compound inhibited lipid peroxidation in emulsions of arachidomic acid containing haemoglobin as initiator, as well as photooxidation of mitochondrial lipids. It was almost as reactive as vitamin E.

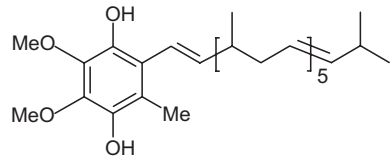
Some of the phenolic compounds isolated from plants having antioxidant properties are given below.



BUTEIN, 19



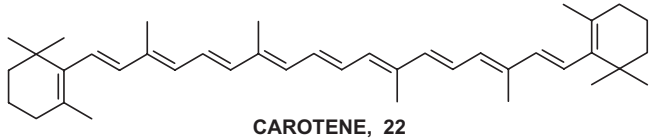
PENTAHYDROXYDIHYDROCHALCONE, 20



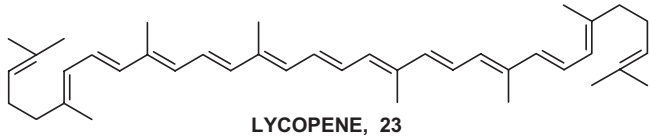
UBIQUINOL. 21

Phenolic compounds have attracted more and more attention as potential agents for preventing and treating many oxidative stress-related diseases. Several studies showed that phenolic compounds were the main antioxidant ingredients in several medicinal plants [25].

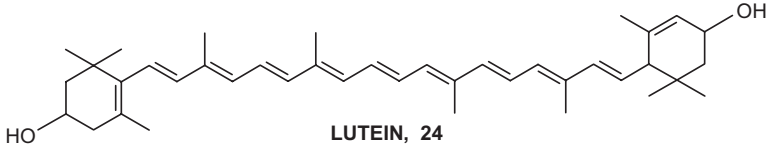
(f) *Carotenoids*: the carotenoids are yellow or orange pigments which are widely distributed in plants, such as sweet potato, carrots, spinach, red pepper, tomatoes, and broccoli. Several forms exist, including β-*carotene*, **22**, *lycopene*, **23** and *lutein*, **24**. The carotenoids act as photosensitizers in conjunction with chlorophyll; when chlorophyll is absent, e.g., in fungi, then the carotenoids mainly are responsible for colour.



CAROTENE, 22



LYCOPENE, 23



LUTEIN, 24

In the search for sources of NAO, in the last few years some medicinal plants have been extensively studied for their antioxidant activity and radical scavenging activity [26–30]. The antioxidant activities of the methanol extracts of selected varieties and parts of garlic and onion were determined by two methods: inhibition of lipid peroxidation induced by *tert*-butyl hydroperoxide in isolated rat hepatoctes and scavenging activities against DPPH radical [31]. In order to find new sources of NAO, antioxidant activities of 40 medicinal plants associated with prevention and treatment of cardiovascular and cerebrovascular diseases were recently evaluated using ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) assays [32]. Antioxidant activities of six rhizomes of Zingiberaceae family were determined by DPPH method and β-carotene-linoleic acid method [33]. The total antioxidant activities of extracts and compounds from the roots of *Smilax lanceaefolia* which is used as folk medicine for stomach ache and rheumatic complaints were recently determined using DPPH method [34,35].

CONCLUSION

Natural antioxidants are already exploited commercially either as antioxidant additives or as nutritional supplements. Also many other plant species have been investigated in the search for novel antioxidants, but generally there is still a demand to find more information concerning the antioxidant potential of plant species as they are safe and also bioactive.

REFERENCES

[1] E.R. Stadtman, R.L. Levine, Ann. N.Y. Acad. Sci. 899 (2000) 191.
[2] S. Yia-Hertuala, Ann. N.Y. Acad. Sci. 874 (1999) 134.
[3] L.J. Marnett, Carcinogenesis 21 (2000) 361.
[4] C.S. Foote, In: W.A. Pryor (Ed.), Free Radicals in Biology, Academic Press, New York, 1976, pp. 85.
[5] H.H. Wasserman, R.W. Murray, Singlet Oxygen. Academic Press, New York, 1979.
[6] A.A. Gorman, M.A.J. Rodgers, Chem. Soc. Rev. 10 (1981) 205.
[7] C.S. Foote, In: A.P. Autor (Ed.), The Pathology of Oxygen, Academic Press, New York, 1982, pp. 25.
[8] C.S. Foote, F.C. Shook, R.B. Abakeru, Methods Enzymol. 105 (1984) 36.
[9] B. Halliwell, Chloroplast Metabolism, Oxford University Press, Oxford, 1981.
[10] U. Takahama, M. Nishimura, Plant Cell Physiol. 16 (1975) 737.
[11] U. Takahama, Plant Cell Physiol. 19 (1978) 1565.
[12] D.J. Betteridge, Metabolism 49 (2000) 3.
[13] B. Halliwell, Free Radic. Res. 31 (1999) 261.
[14] G.K. Jayaprakasha, R.P. Singh, K.K. Sakariah, Food Chem. 73 (2001) 285.
[15] N. Ramarathnam, H. Ochi, M. Takeuchi, Nat. Antioxidants Chem. Health Effects Appl. 76, 1996.
[16] Y.S. Velioglu, G. Mazza, Y.L. Gao, B.D. Oomah, J. Agri. Food Chem. 46 (1998) 4113.
[17] N.C. Cook, S. Samman, Nutr. Biochem. 7 (1996) 66.

[18] B.D. Oomah, G. Mazza, J. Agri. Food Chem. 7 (1994) 1746.
[19] R. Amarowicz, U.N. Wanasunddara, M. Karamac, F. Shahidi, Nahrung 40 (1996) 261.
[20] A. Matkowski, P. Tasarz, E. Szpula, J. Med. Plant Res. 2 (2008) 321.
[21] M.S. Al-Saikhan, L.R. Howard, L.C. Miller Jr., J. Food Sci. 60 (1996) 341.
[22] G. Yen, P.D. Duh, Am. Oil Chem. Soc. 72 (1995) 1065.
[23] P. Veeru, M.P. Kishor, M. Meenakshi, J. Med. Plant Res. 3 (2009) 608.
[24] A. Rafat, K. Philip, S. Muniandy, J. Med. Plant Res. 4 (2010) 197.
[25] H.Y. Liu, N.X. Qiu, H.H. Ding, R.Q. Yao, Food Res. Int. 41 (2008) 363.
[26] C. Desmarchelier, G. Ciccia, J. Coussio, Stud. Nat. Prod. Chem. 22 (2000) 343.
[27] G.R. Schinella, H.A. Tournier, J.M. Prieto, P.M. Buschiazzo, J.L. Rios, Life Sci. 70 (2002) 1023.
[28] T.J. Vanderjagt, R. Ghattas, D.J. Vanderjagt, M. Crossey, R.H. Glew, Life Sci. 70 (2002) 1035.
[29] K.R. Sini, B.N. Sinha, M. Karpagavalli, Curr. Bot. 1 (2010) 13.
[30] I. Parejo, F. Viladomat, J. Bastida, A.R. Romero, G. Saavedra, M.A. Murcia, A.M. Jimenez, C. Codina, Life Sci. 73 (2003) 1667.
[31] A.M. Nuutila, R.P. Pimia, M. Aarni, K.M.O. Caldentey, Food Chem. 81 (2003) 485.
[32] R.Y. Gan, X.R. Xu, F.L. Song, L. Kuang, H.B. Li, J. Med. Plant Res. 4 (2010) 2438.
[33] S.L. Warjeet, P.S. Vankar, V. Tiwari, N. Swapana, Elect. J. Environ. Agric. Food Chem. 5 (2006) 1318.
[34] S.L. Warjeet, D.K. Brajeshwari, Nat. Prod. Res. 24 (2010) 1168.
[35] S.L. Warjeet, D.K. Brajeshwari, S.Y. Rajkumar, Int. Res. J. Pure Appl. Chem. 1 (2011) 1.

Chapter 10

Bioactive Natural Products as Potential Candidates to Control *Aedes aegypti*, the Vector of Dengue

Regina Geris[†], Paulo Roberto Ribeiro[†], Maurício Da Silva Brandão[†], Heloísa Helena Garcia Da Silva[*] and Ionizete Garcia Da Silva[*]

[†]*Instituto de Química, Universidade Federal da Bahia, Salvador – BA, Brazil*

[*]*Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brazil*

INTRODUCTION

Mosquitoes are one of the most important insect groups which act as vectors of pathogens for several human diseases [1], including malaria, filariasis, dengue and dengue hemorrhagic fever, encephalitis, yellow fever and others, especially in the tropical and subtropical areas [2].

The mosquito *Aedes aegypti* (Linnaeus, 1762) is the main vector responsible for dengue and yellow fever, both endemic diseases covering tropical and subtropical regions, mainly in urban and semi-urban areas [3,4]. Although yellow fever has been reasonably brought under control, no vaccine is available against dengue [5]. So far, the only method of controlling or preventing dengue virus transmission is to combat the vector mosquitoes by reducing the adult population densities [6]. Vector control is implemented using environmental management and chemical methods. Community-based programmes have stimulated people to dispose their solid waste properly and keep their water containers covered in order to avoid access by egg-laying female mosquitoes [6].

Chemicals have been used to control *Ae. aegypti* since the beginning of the twentieth century and the current methods for applying insecticides include larvicide application, perifocal treatment and space spraying [7]. Four larvicides can be used to treat containers that hold drinking water, which have extremely low mammalian toxicity: the organophosphorus temephos (**1**), the insect growth regulators

Bioactive Natural Products (Part Q), Vol. 37. http://dx.doi.org/10.1016/B978-0-444-59514-0.00010-9

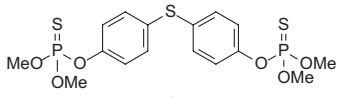
methoprene (**2**) and pyriproxyfen (**3**) and also the biological agent BTI (*Bacillus thuringiensis israelensis* H-14); among them, temephos (at the concentration of 1% sand granules) and BTI are the most commonly used larvicides [7,8]. Perifocal treatment involves the use of hand or power sprayers to apply formulations of insecticides [malathion (**4**), fenitrothion (**5**), fenthion (**6**), and some pyrethroids such as pyretrins I (**7**) and II (**8**), permethrin (**9**) and cypermethrin (**10**)] as a spray to larval habitats and peripheral areas, as well as non-potable water in containers [7].

The need to minimize the dependency on chemical insecticides or their indiscriminate use allied to development of vector resistance to the commonly used insecticides, including temephos, have been directed for the search of alternative methods for vector control. These new strategies could be mainly used as routine in control programs during the periods of little or no dengue virus activity.

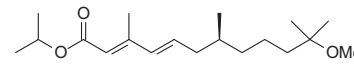
Biopesticides, a term which includes microbial organisms, nematodes, secondary metabolites from plants and microorganisms, insect pheromones, genes, insect predators and parasites, received much attention for being environmentally friendly [9] and potentially used as leads for a variety of insect control agents, such as pyrethroids earlier obtained from pyrethrum flowers *Chrysanthemum cinerariaefolium* (Asteraceae) [10].

Natural products represent a vast structural and functional diversity of molecules and they remain to be the best source and inspiration for the discovery of new drugs and molecular targets. Plants extracts and their secondary metabolites have been recognized to have insecticidal activities such as repellent, antifeedant, and growth-regulating properties [5,11,12]. Some compounds produced by microorganisms also show insecticidal activities [13,14].

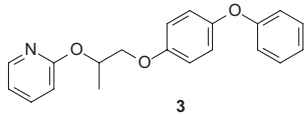
Besides, to diversify the availability of control options, researches have also been driven to find entomopathogenic organisms, including the fungi *Metarhizium anisopliae* and *Beauveria bassiana* and the bacteria *B. thuringiensis* var. *israelensis*, aiming the regulation of insect populations.



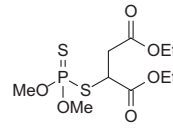
1



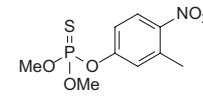
2



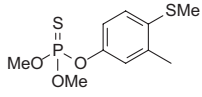
3



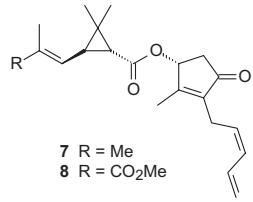
4



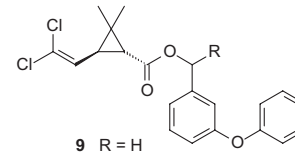
5



6



7 R = Me
8 R = CO_2Me



9 R = H
10 R = CN

Scope of the Review

The review highlights the secondary metabolites produced by living organisms, including plants, fungi, bacteria (actinomycetes and cyanobacteria), lichens, and algae which were active against the mosquito *Ae. aegypti*. These insecticidal compounds display a huge range of structural diversity comprising fatty acids and derivatives, quinones, polyketides, non-ribosomal peptides, phenylpropanoids, coumarins, flavonoids, terpenoids, alkaloids, which are described herein. Some known biosynthetic pathways are presented as well. The related insecticidal activities, such as adulticidal, mosquitocidal, larvicidal and ovicidal, as well as the mode of action of some compounds are also summarized. LC value units were expressed in the text as described in their original papers. However, for convenience, the LC values presented in the tables were all expressed in ppm in order to provide a basis for comparison of their potencies.

Due to the extensive literature reviews about bioactive extracts and essential oils from plants against *Ae. aegypti* [15–21] we limited this review to bioactive secondary metabolites isolated from or identified in bioactive fractions of plants. Biocontrol agents were briefly commented since they can secret enzymes, proteinaceous toxins and secondary metabolites and can also be a promising source of bioactive compounds against *Aedes* mosquito.

The literature (*via* Chemical Abstracts, Web of Science, and PubMed databases) was searched for natural products as candidates to control *Ae. aegypti*, using the following search terms: natural product(s), bioactive compound(s), bioactive metabolites, mosquitocide(s), mosquitocidal, adulticidal, larvicidal, larvicide(s), insecticidal, insecticide(s), plant, fungi, fungus, bacteria, actinomycetes, algae, and lichen, added to the terms *Aedes* or *Ae. aegypti*.

Dengue

Dengue is a disease caused by several arthropod-borne viruses that affects infants, young children and adults, and is characterized by fever, myalgia or arthralgia, rash, leucopenia and lymphadenopathy, but rarely causes death [6,22]. However, dengue haemorrhagic fever (DHF) is a severe and potentially fatal disease characterized by high fever, enlargement of the liver, circulatory failure that leads, in harsh cases, to a protein-losing shock syndrome (dengue shock syndrome, DSS) [6,22].

Dengue viruses encompass four related serogroups that cause very similar disease in humans, named dengue-1 (DENV-1), dengue-2 (DENV-2), dengue-3 (DENV-3), and dengue-4 (DENV-4) [23]. They are members of the *Flavivirus* genus, family Flaviridae, which is also termed "arboviruses" or arthropod-borne viruses since they are transmitted between vertebrate hosts by mosquitoes [23]. Since they are antigenically and genetically distinct, an infection by one of dengue serotypes can immunize only for that specific serotype. It theoretically means that an individual can be infected by the four serogroups, although third infections are very rare and fourth infections have never been reported [24].

Uninfected female *Aedes* mosquitoes generally acquire the virus while feeding on the blood of an infected person, becoming infected and transmitted it to humans by their bites [6]. *Ae. aegypti* is the most important vector of dengue viruses, although *Ae. albopictus* and *Ae. polynesiensis* can act as vectors in some localities [24]. After virus incubation for 8–10 days, an infected mosquito is capable, during probing or blood feeding, of transmitting the virus for the rest of its life, approximately 6–8 weeks [6].

Female mosquitoes are very sensible and they can disrupt the feeding process at the slightest movement and return to the same or a different person later; if the females are infected they may transmit dengue virus to several individuals in a short period of time even if they probe without feeding [25]. The virus circulates in the peripheral blood of infected humans for 2–7 days, at approximately the same time that they have a fever [6]. The transmission cycle can propagate if an unaffected mosquito bites the ill person and subsequently transmit the virus to the other unaffected persons.

In the last 50 years dengue has become the most propagated disease in countries around the intertropics region and its incidence is increasing [6,24]. According to the WHO estimative, 2.5 billion people are at risk to catch dengue with the possibility of 50 million dengue infections worldwide every year [6,24]. The spread of dengue is attributed to expanding geographic distribution of the four dengue viruses and their mosquito vector and it is found mainly in tropical and subtropical regions, predominantly in urban and semi-urban areas [6].

There is no vaccine to protect against dengue. Development of a vaccine is not simple since it must immunize against all four viruses types to be effective, i.e., only a tetravalent vaccine will be acceptable because there is no cross-protection between the four DENV serotypes [6,24]. Besides, dengue virus grows poorly in cell culture, and there is the need of a reliable animal model for test [24]. In this context, two vaccine candidates have advanced to evaluation in human subjects in countries with endemic disease, and several potential vaccines are in earlier stages of development [6]. Guy *et al*. reported the development of a tetravalent chimeric dengue vaccine by ChimeriVax technology and preclinical and clinical tests revealed safety and immunogenicity against all four DENV serotypes. The process is in the large-scale efficacy trials [26].

Aedes aegypti Mosquito

Dengue vectors are members of the genus *Aedes*, subgenus *Stegomya*, family Culicidae, and order Diptera [27]. *Aedes aegypti* is the most important vector distributed in several endemic countries, while *Ae. albopictus* and *Ae. polynesiensis* are endemic species in Asia [8]. *Aedes aegypti* and *Ae. albopictus* are both container-breeders, preferentially in relatively unpolluted water. The former is predominant in man-made artificial containers (urban areas) while the latter is found in natural habitats such as tree holes, rock pools and leaf axils [27,28].

Aedes aegypti is a holometabolous insect, i.e., the life cycle encompasses a complete metamorphosis with an egg, larvae (first, second, third, and fourth instars), pupae, and adult stage, which can be completed within 1.5–3 weeks [29]. Larval and pupae stages are developed in aquatic phase while eggs and adults are terrestrial phase. Adults are recognized by their external morphologic aspects [30]: their bodies are predominantly dark, contrasting with silvery-white scales presented in the clipeo and in the basis of their anthems. Moreover, dorsal thorax contains two external sinuous lines and two internal retilinea lines with silvery scales resembling the musical instrument lyre (Fig. 1) [30].

The *Aedes* females need iron obtained from a vertebrate blood meal (usually human) to obtain nutrients for egg maturation [31]. Two days after blood digestion, the ovaries contain mature eggs [32] and, after mating, the females deposit their eggs on damp surfaces, mainly where rainwater can be stored (such as

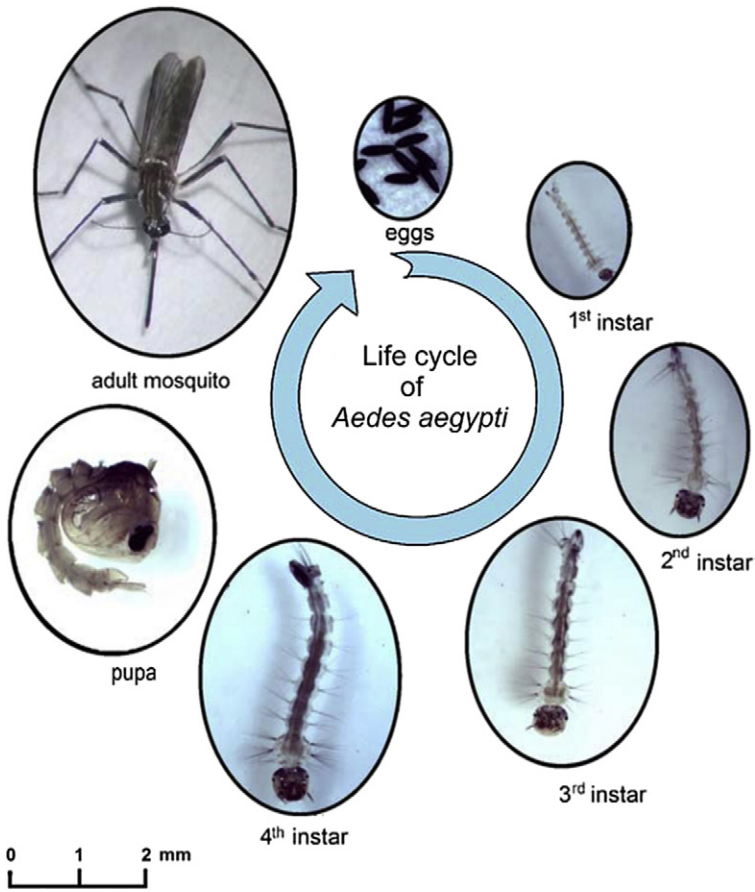


FIGURE 1 Life cycle of *Aedes aegypti*.

barrels, flower vases, tyres, tanks, etc.). After 2–7 days, these developed eggs hatched leading to the fourth sequencing larval stages: first (2mm, 2.5 ± 0.1 days), second (3mm, 0.9 ± 0.1 days), third (4mm, 1.2 ± 0.1 days) and fourth (5mm, 2.1 ± 0.3 days) instars, taking approximately 8 days (Fig. 1) [33]. Then, larval enter the pupal stage (5mm) and, after 2–3 days, they transform into adult mosquito (male 27.14 days and females 42.25 days), initiating a new life cycle.

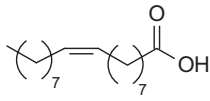
Insectides can act on mosquitoes at different modes. Organochlorines and synthetical pyrethroids act on sodium channels of adult mosquito [6], while natural derived insecticides lead to death by destroying mesentero and expulsing perithrophic matrix of larvae [34–37]. Growth-inhibitor insecticides block initial stages ecdise [38].

NATURAL INSECTICIDES DERIVED FROM PLANTS

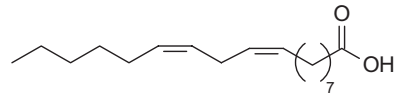
Secondary Metabolites Derived from Plants

Fatty Acids and Derivatives

Oleic (**11**) and linoleic (**12**) acids have been reported as larvicidal compounds against fourth instar larvae of *Ae. aegypti*. These compounds were isolated from *Dirca palustris* (Thymelaeaceae) [39] and *Citrullus colocynthis* (Linn.) Schrad (Curcubitaceae) [40]. Larvicidal bioassay performed by Ramsewak *et al.* indicated an LC_{50} of 100ppm for both compounds [39], while bioassay performed by Rahuman *et al.* revealed an LC_{50}/LC_{90} values of 8.8/35.39ppm for **11** and 18.2/96.33ppm for **12** [40]. Moreover, oleic acid (**11**) exhibited repellent activity against female mosquitoes at 30min after treatment at a dosage of 0.4mg/cm^2 [41].



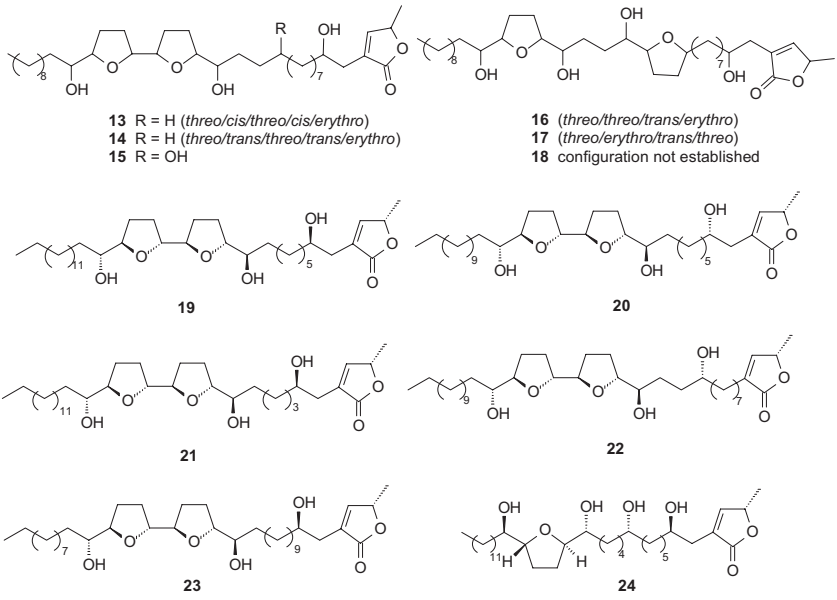
11



12

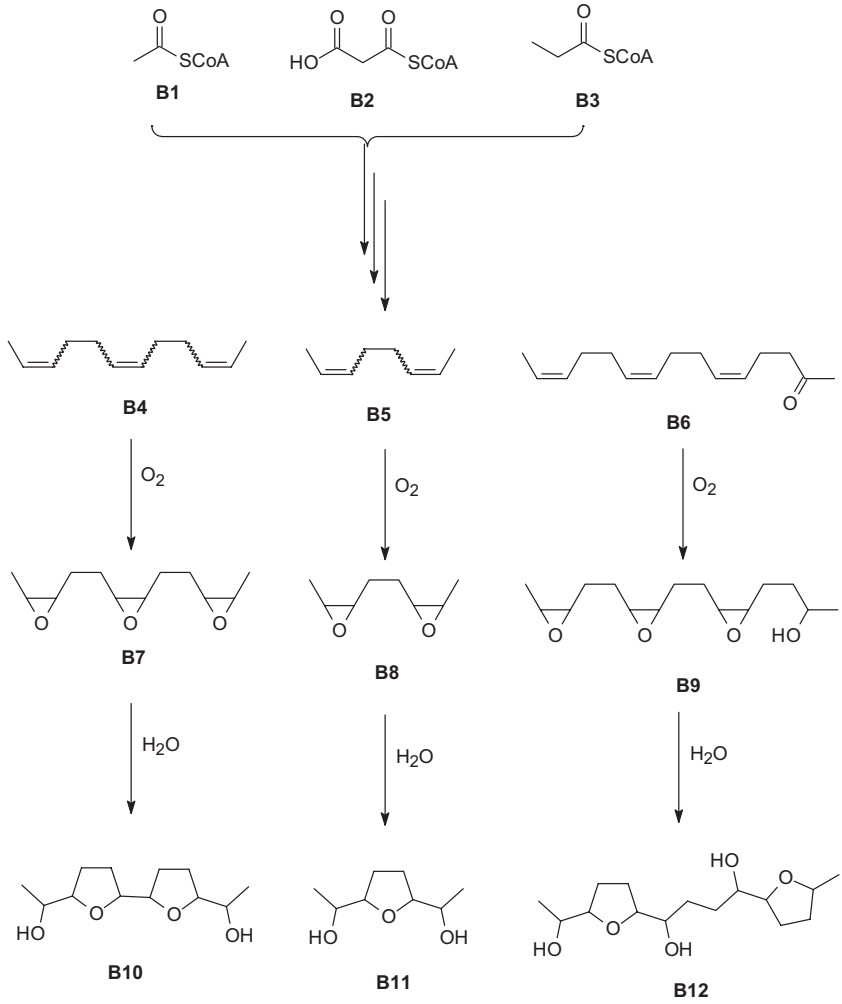
The annonaceous acetogenins (derivatives of C35 or C37 fatty acids chain) rolliniastatin (**13**), bullatacin, (**14**), purpureacin 2 (**15**), cherimoline (**16**), sylvaticin (**17**), and purpureacin 1 (**18**) were isolated through activity-directed fractionation of the larvicidal dichloromethane extract from leaves of *Annona purpurea* L. (Annonaceae) (LC_{100} 10ppm, 24h) and identified on the basis of spectral evidences [42]. These compounds showed larvicidal activity against second instar of *Aedes* mosquito with LC_{100} values of 0.2, 0.3, 2.0, 1.0, 2.0 and 1.0ppm, respectively, to compounds **13–18**, being acetogenins **13** and **14** the most toxic to the larvae [42]. In addition, the acetogenins longimicins A (**19**), B (**20**), C (**21**) and D (**22**) were obtained by bioactivity-directed fractionation of the ethanol extract of *Asimina longifolia* (Annonaceae) and their structures, stereochemistries (*threo/trans/threo/trans/threo*), as well as the positions of their THF ring systems, were determined through 1D- and 2D-NMR experiments

and EIMS analyses [43]. Their potential as larvicides were evaluated against third or early fourth instar larvae of *Ae. aegypti*, in addition to the known asimicin (**23**) [44], exhibiting LC_{50} values of 141.0, 30.4, 75.7, 8.29 and 3.04μg/mL, respectively, to **19**, **20**, **21**, **22**, and **23** [43]. The lower potencies for these acetogenins compared with **23** should be the position of the adjacent bis-THF ring moiety that is essential for maximization of the bioactivities among them [43]. Moreover, annonacin (**24**), isolated from *Annona densicoma* (Annonaceae) [45], was toxic to third instar larvae with LC_{50} of 9.5μg/mL and LC_{90} of 22.3μg/mL [46].



Structures of these annonaceous acetogenins strongly suggest their polyketide origin [47,48]. The precursors could have been assembled by the linear combination of acyl thioesters (with two and three carbon units like acetic acid and propanoic acid) in a similar way for fatty acids biosynthesis *via* acetyl-CoA (**B1**), malonyl-CoA (**B2**), and propanyl-CoA (**B3**). Epoxidation of triene (**B4**), diene (**B5**) or triene ketones (**B6**) intermediates, followed by ring openings and closures initiated by water attacking the epoxides would produce the tetrahydrofuran rings of acetogenins (**B10–B12**), as shown in Scheme 1 [47,48].

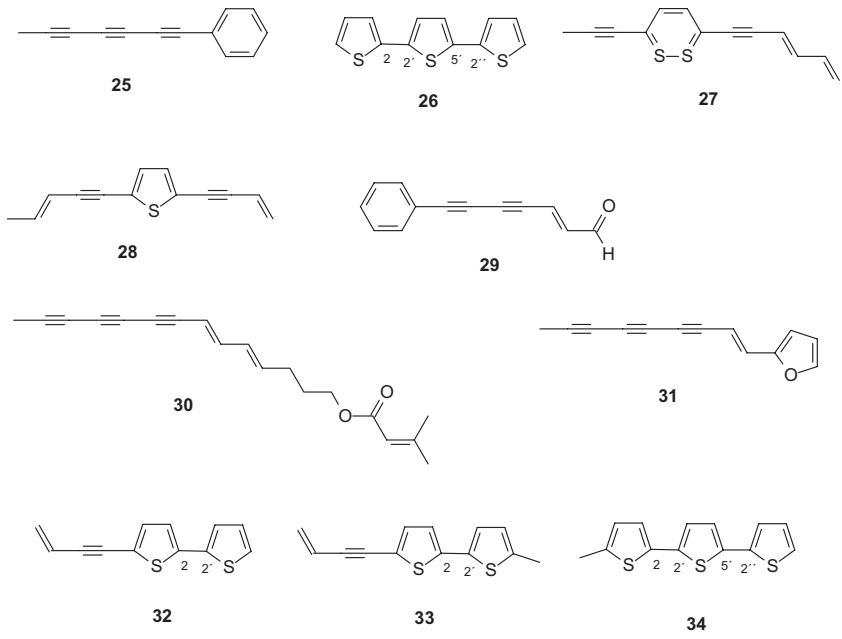
Fourteen natural polyacetylenes and their thiophene derivatives were tested on several stages of the *Aedes* larvae under an artificial source of ultraviolet light in order to search for insect photosensitizer compounds [49]. Six of 14 (**25–30**) caused 100% mortality against first instar at a concentration of 0.5mg/mL, while **31** led to the 67–99% mortality. Among them, **25**, **26**, and **30** also killed second instar, and a 100% mortality of fourth instar was obtained only with the tiophene



SCHEME 1 Hypothetical biosynthesis pathway of annonaceous acetogenins. Adapted from [47] and [48].

derivative **26** (α-terthienyl). Moreover, compounds **26** and the furanoacetylene **27** showed LC_{50}/LC_{90} values of 0.019/0.039ppm and 0.079/0.160ppm, respectively, against the fourth larval stage [49,50]. α-Terthienyl was first isolated from the yellow petals of *Tagetes erecta* (Asteraceae) [51].

Additional tiophene derivatives 5-(but-3-ene-1-ynyl)-2-2'-bithiophene (**32**), 5-(but-3-ene-1-ynyl)-5'-methyl-2-2'-bithiophene (**33**), and 5-methyl-2,2',5',2''-terthiophene (**34**) were identified as bioactive components of fraction from floral extracts of *T. minuta*. This fraction showed toxicity to third larval stage of *Aedes* larvae (LC_{50} 3.9ppm) and to adults of *Ae. aegypti* and *Anopheles stephensi* [52].



Toxicity of α-terthienyl (**26**) is based on the production of a toxic singlet oxygen by a type II photodynamic process leading to damages to the nervous, respiratory and digestive systems of the larvae [53,54]. Experiments using ^{35}S-Na_2SO_4, ^{35}S-*L*-cystein and ^{14}C feeding with *Tagetes patula nana* and *T. erecta* suggested a biosynthetic origin of **26** from bithienylbutadiine (**B13**), as shown in Scheme 2 [55].

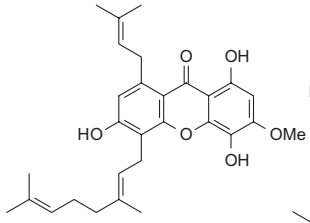
SCHEME 2 Proposed biosynthetic precursor for the α-terthienyl **26** [55].

Xanthones

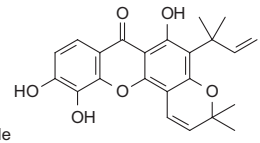
Some crude extracts from plants belonging to the Clusiaceae family were evaluated against third instar of *Aedes* mosquito. From the bioactive crude extract of *Garcinia cuneifolia* bark was isolated the cuneifolin (**35**), a xanthone that possess a ring substituted by a prenyl and a geranyl group [56]. The pyranoxanthones inophyllin B (**36**) and brasilixanthone (**37**) were obtained after fractionation of crude extract from roots of *Calophyllum inophyllum* [57]. These three xanthones showed to be toxic to larvae of *Ae. aegypti*. Further investigation on

the extract from roots of this plant resulted in the isolation of inophyllin A (**38**) which showed LC_{50} of 75–100μg/mL [58].

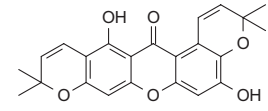
Chemical studies on the stem of *Garcinia mangostana* afforded the isolation and identification of seven xanthones from which α-mangostin (**39**), β-mangostin (**40**) and mangosharin (**41**) were evaluated for their larvicidal activities [59]. Among them, only xanthone **39** showed activity against the third larval stage with an LC_{50} of 19.4ppm [59].



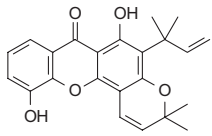
35



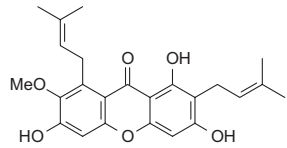
36



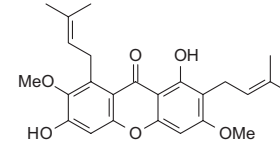
37



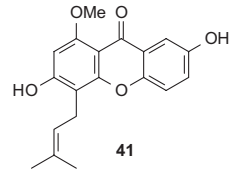
38



39



40

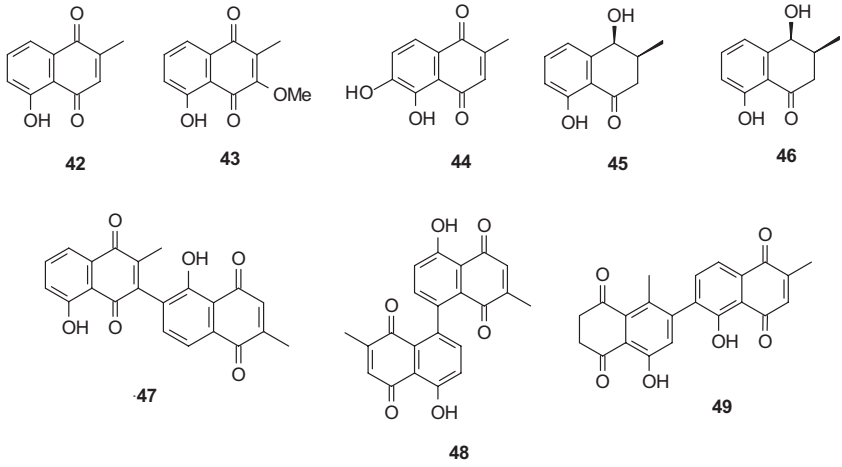


41

Quinones

A bioassay-guided phytochemical investigation of chloroform extract from *Plumbago capensis* Thunb (Plumbaginaceae) roots led to the isolation of eight naphthoquinone derivatives whose structures were established by IR, MS, 1D- and 2D-NMR spectroscopic techniques and comparison with literature data [60]. They are plumbagin (**42**), 3-*O*-methyl droserone (**43**), 6-hydroxy plumbagin (**44**), isoshinanolone (**45**), isoplumbagolone (**46**), chitranone (**47**), maritinone (**48**), and chitranane (**49**). Among these compounds, isoshinanolone (**45**), plumbagin (**42**), and 6-hydroxy plumbagin (**44**) have showed highest larvicidal activities against fourth instar larvae of *Ae. aegypti*, with LC_{50} (and LC_{90}) values of 1.26μg/mL (LC_{90} = 4.10μg/mL), 5.43μg/mL (LC_{90} = 6.56μg/mL), and 13.64μg/mL (LC_{90} = 19.28μg/mL), respectively. Compounds **43**, **48**, and **49** showed LC_{50} values (and LC_{90}) of 31.47μg/mL (55.72μg/mL), 40.66μg/mL (LC_{90} = 53.87μg/mL), and 31.21μg/mL (LC_{90} = 42.72μg/mL), respectively, while **46** and **47** were not active at highest concentration tested (60μg/mL) [60]. Sreelatha *et al*. have

suggested that the presence of a reduced quinine ring (ring B), a hydroxyl group at fourth position and a methyl group at third position appear to play an important role on the mosquitocidal activity of these naphthoquinone derivatives.

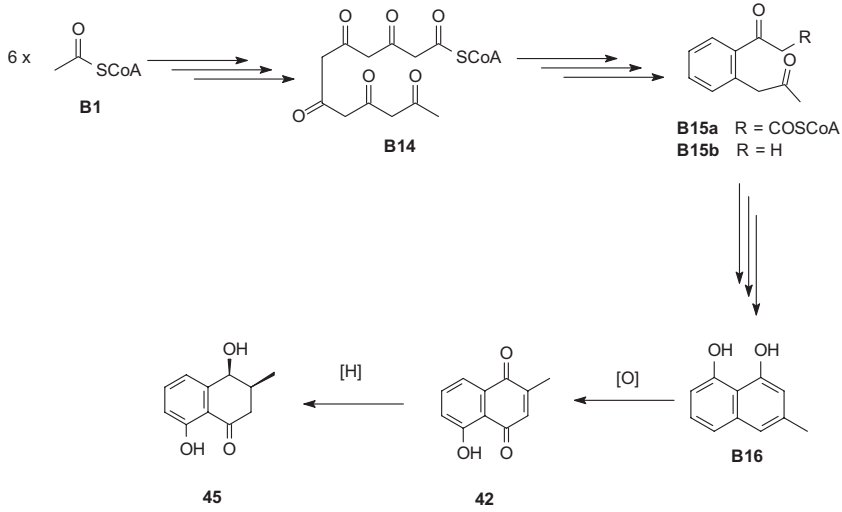


Feeding experiments with ^{13}C-labelled acetate established the polyketide origin to naphthoquinones plumbagin (**42**) and isoshinanolone (**45**) (Scheme 3). The open-chained hexaketide precursor (**B14**) should undergo to cyclization (*via* **B15**a or **B15b**) to produce the naphthalene skeleton (**B16**). Further oxidation gives **42** which under reduction step led to **45** [61].

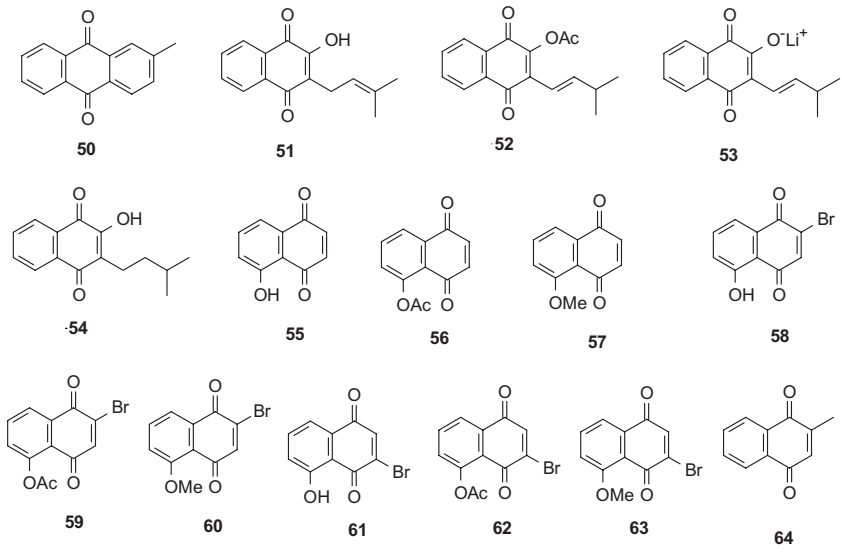
Tectoquinone (**50**), isolated from the methanolic extract of *Cryptomeria japonica* (Taxodiaceae) sapwood, exhibited larvicidal activity against fourth instar larvae of both *Ae. aegypti* and *Ae. albopictus*. The LC_{50} (and LC_{90} values) of **50** after 24h were 3.3μg/mL (LC_{90} = 8.8μg/mL) against *Ae. aegypti* larvae and 5.4μg/mL (LC_{90} = 26.9μg/mL) against *Ae. albopictus* larvae [62].

Lapachol (**51**), a 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone, obtained from fractionation of *Cybistax antisyphilitica* (Martius) Martius (Bignoniaceae) was active against third instar larvae of *Aedes* showing LC_{50} value of 26.3μg/ml [63]. In addition, several lapachol derivatives were synthesized and tested against fourth instar larvae [64]. LC_{50}/LC_{90} values of these compounds were 9.64/16.154ppm to isolapachol acetate (**52**), 3.78/7.06ppm to isolapachol lithium salt (**53**), 4.745/9.062ppm to dihydrolapachol (**54**), 3.587/6.641ppm to 5-hydroxy-1,4-naphthoquinone (**55**), 4.553/7.993ppm to 5-acetoxy-1,4-naphthoquinone (**56**), 7.919/10.833ppm to 5-methoxy-1,4-naphthoquinone (**57**), 1.391/2.501ppm to 2-bromo-5-hydroxy-1,4-naphthoquinone (**58**), 1.170/4.550ppm to 2-bromo-5-acetoxy-1,4-naphthoquinone (**59**), 9.703/16.999 ppm to 2-bromo-5-methoxy-1,4-naphthoquinone (**60**), 0.873/1.417ppm to 3-bromo-5-hydroxy-1,4-naphthoquinone (**61**), 7.287/10.445ppm to 3-bromo-5-acetoxy-1,4-naphthoquinone (**62**), 5.752/12.453ppm to 3-bromo-5-methoxy-1,4-naphthoquinone (**63**), and 14.952/23.065ppm to 2-methyl-1,4-naphthoquinone

(**64**), in addition to temephos (**1**) that exhibited LC_{50}/LC_{90} values of 1.4999/4.799 [64]. Comparative analysis indicated that compounds **58**, **59**, and **61** are more potent than temephos, suggesting, therefore, that the presence of a bromine group in these compounds enhances the activity. Moreover, it seems that a free hydroxyl group is an essential condition for larvicidal the observed activity in **55** [64].

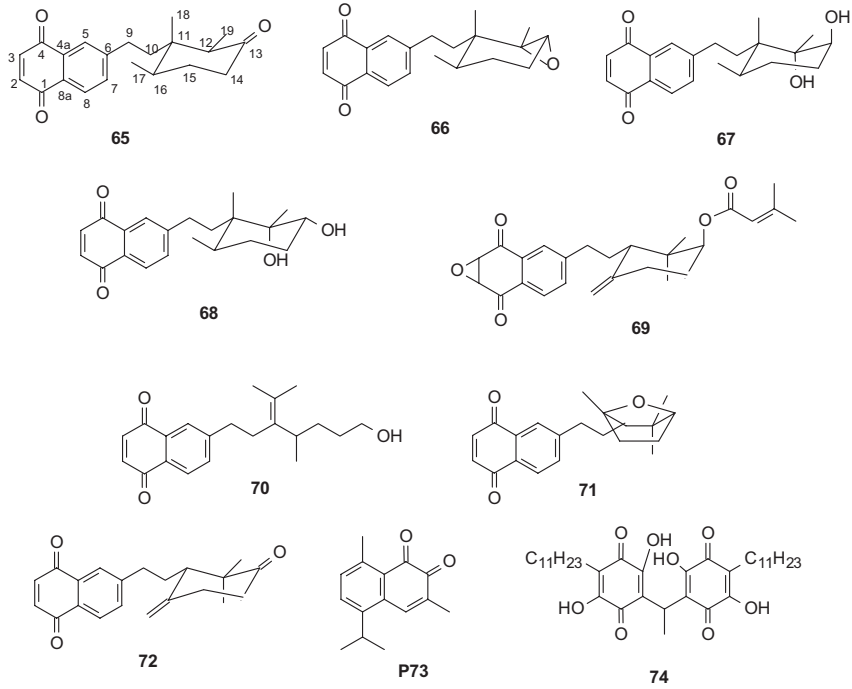


SCHEME 3 Proposed biosynthetic pathway to plumbagin (**42**) and isoshinanolone (**45**). Adapted from [60].

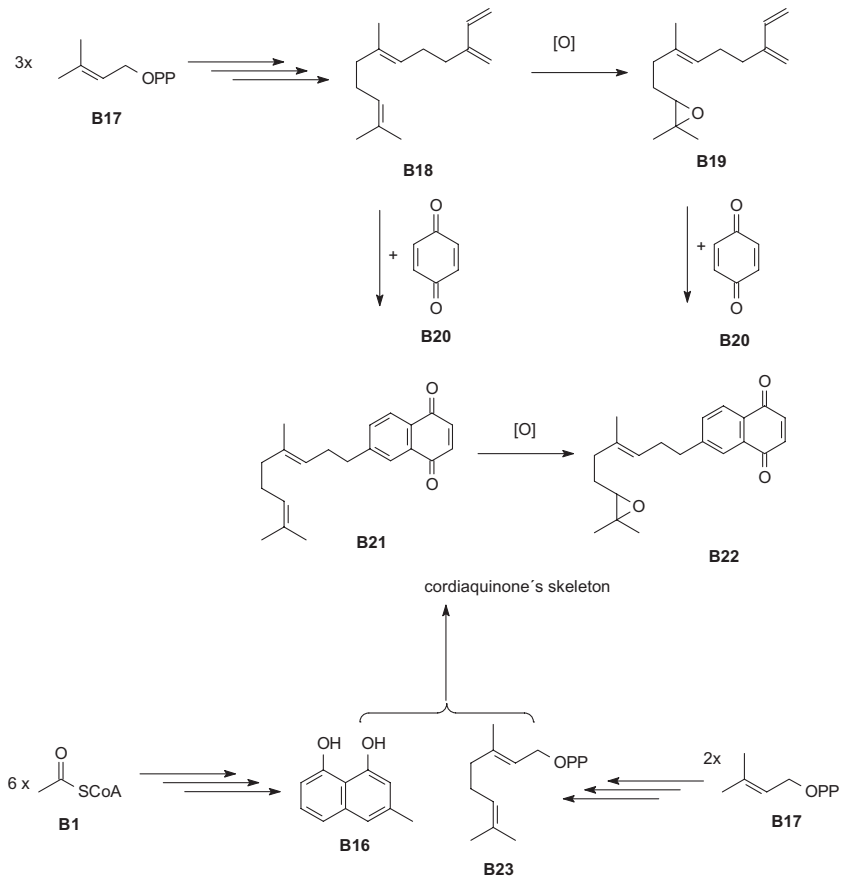


The meroterpenoids naphthoquinones cordiaquinones B (**65**), E (**66**), F (**67**), G (**68**), and H (**69**) were isolated from dichloromethane extract of *Cordia linnaei* (Boraginaceae) [65], and cordiaquinones A (**70**), J (**71**), and K (**72**) in addition to **65** were obtained from *Cordia curassava* [66]. Larvicidal potency of these 6-substituted naphthoquinones was determined against second instar larval *Aedes* mosquito and the minimal concentration of compounds required to kill all the larvae after 24h were 25, 12.5, 50, 25, 12.5, 25, 12.5μg/mL, respectively to **65**, **66**, **67**, **68**, **70**, **71**, and **723**. Compound **69** was not tested [65,66].

Sesquiterpene *ortho*-quinone mansonone C (**73**) was obtained from dichloromethane extract of *Mansonia gagei* Drumm (Sterculiaceae) heartwood and exhibited larvicidal activity against larvae of *Ae. aegypti*, with a minimal concentration of the compound required to kill all the larvae in 24h of 6.25ppm [67]. In addition, methyl vilangin (**74**), obtained from air-dried berries of *Embelia schimperi* (Myrsinaceae), was found to be lethal against second instar larvae of *Ae. aegypti* by disrupting the process of metamorphosis and then leading to death (100μg/mL of **74** caused 70% mortality of second instar stage) [68].



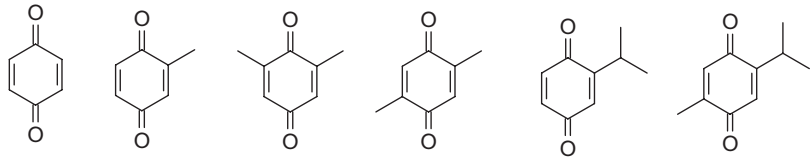
There is no direct experimental evidence for the biosynthetic origin of cordiaquinones. However, Arkoudis and Stratakis suggested a Diels–Alder reaction of the naturally occurring β-farnesene (**B18**) or epoxy-β-farnesene (**B19**) with benzoquinone (**B20**) to give cordiaquinone skeleton (**B21** or **B22**), which



SCHEME 4 Hypothetical biosynthetic pathways for cordiaquinones [61,69,70].

in turn led to cordiaquinones by acid-catalyzed cyclization (Scheme 4) [69]. They also indicated an alternative route where naphthoquinone moiety of cordiaquinones (**B16**) could arise from a polyketide pathway, similar to plumbagin (**42**) [61] and anthraquinones [70].

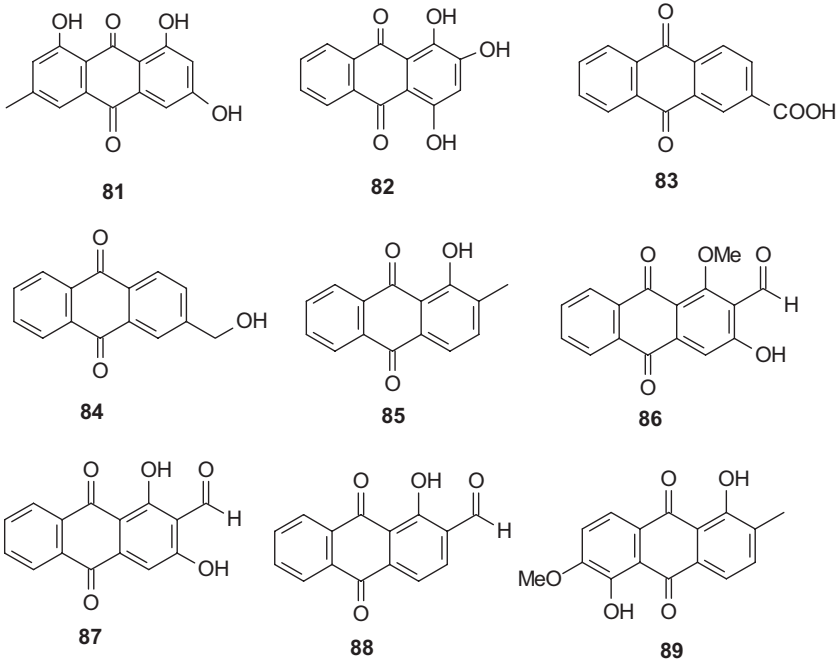
Since *p*-benzoquinones and derivatives show larvicidal activity against *Ae. aegypti* larvae, six structurally related compounds were prepared in order to investigate such structural modifications that can increase their potencies against third instar larval stage. They are *para*-benzoquinone (**75**), 2-methyl-benzoquinone, 2-methyl-*para*-benzoquinone (**76**), 2,6-dimethyl-*para*-benzoquinone (**77**), 2,5-dimethyl-*para*-benzoquinone (**78**), 2-isopropyl-*para*-benzoquinone (**79**), and thymoquinone (**80**) and their respective LC_{50} values were 90, 61, 42, 57, 33, 48μg/mL [71]). The unsubstituted *para*-benzoquinone (**75**) was the compound that exhibit the lowest activity (LC_{50} = 90.0μg/mL) while compounds containing alkyl groups showed lower LC_{50} values; among this, it could be suggested that lipophilicity may be related to the larvicidal activity [71].



Anthraquinones

Emodin (**81**) was isolated from the methanol crude extract from seeds of *Cassia obtusifolia* (Fabaceae) and evaluated against early fourth instar larvae together with the commercially available hydroxyanthraquinones alizarin, danthron, quinizarin, and purpurin (**82**), from which only **81** and **82** exhibited larvicidal activity with LC_{50} of 1.9 and 19.6μg/mL, respectively [72]. In addition, anthraquinone-2-carboxilic acid (**83**) and (**84**) were active with LC_{50}/LC_{90} of 16.3/25.0 and 15.4/23.7ppm, respectively [62].

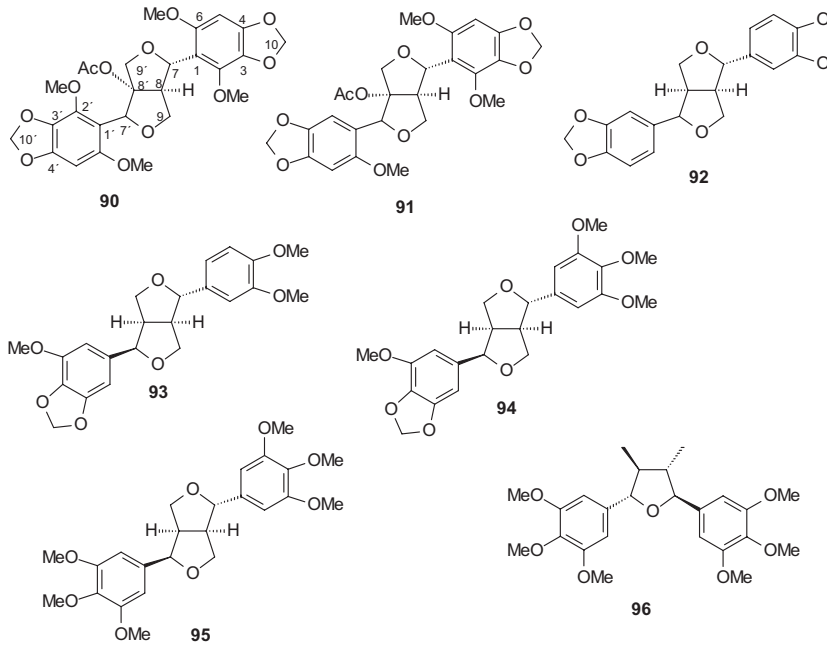
Five anthraquinones were obtained from *Morinda citrifolia* (Rubiaceae) roots and they were identified by spectroscopic analyses including NMR, MS, and IR as 1-hydroxy-2-methylanthraquinone (**85**), damnacanthal (**86**), nordamnacanthal (**87**), 2-formyl-1-hydroxyanthraquinone (**88**), and morindone-6-methyl-ether (**89**) [73]. **85** and **86** exhibited LC_{50} of 1.8 and 7.4μg/mL, and LC_{90} of 2.9 and 16.5μg/mL, respectively, while compounds **87**, **88**, and **89** gave activity at concentrations superior to 15μg/mL [73].



Lignans and Neolignans

When chloroform fraction obtained from the methanol extract of *Phryma leptostachya* var. *asiatica* (Verbenaceae) roots was examined against third instar larvae *Ae. aegypti*, it was observed that the fraction caused toxicity to mosquito larvae at a concentration of 50ppm after 24h exposure [74]. Subsequent studies afforded the isolation of two lignans, leptostachyol acetate (**90**) and 8'-acetoxy-2,2',6-trimethoxy-3,4,4',5'-dimethylenedioxyphenyl-7,7'-dioxabicyclo-[3.3.0]-octane (**91**), which were identified by means of spectroscopic methods. Compound **90** exhibited an LC_{50} value of 2.1ppm, while the unsubstituted C6' position leptostachyol acetate analogue (**91**) revealed weak or no activity in 24h at 10ppm [74]. In addition (−)-asarinin (**92**), isolated from the methanolic extract of *Asarum heterotropoides* (Aristolochiaceae) roots, exhibited larvicidal activity (LC_{50} = 10.49ppm) against third instar larvae *Ae. aegypti* [75].

Bioassay-guided fractionation of chloroform extract from leaves of *Piper fimbriulatum* (Piperaceae) resulted in the isolation and identification of three lignans 3,4,5'-trimethoxy-3',4'-methylenedioxy-7,9',7',9-diepoxylignan (**93**), 7-epi-sesartemin (**94**), and diayangambin (**95**) through 1-D and 2-D NMR experiments [76]. Among these, only **93** and **94** have shown an LC_{100} value of 25.0 and 17.6µg/mL, respectively. Besides, grandisin (**96**) obtained from *Piper solmsianum* exhibited toxicity to 50% of first instar larvae at 2.37ppm [77].



Phenylpropenes and Phenolic Derivatives

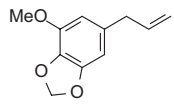
Biological activities of phenylpropenes myristicin (**97**), apiol (**98**), and dill-apiol (**99**), isolated from roots and greens (all aerial plants, including seeds) of *Anethum graveolus* L. (Umbelliferae), were investigated against third instar of *Aedes* larvae exhibiting LC_{50} values of 10.8, 11.5, and 11.0ppm, respectively [78]. A mixture of elemicin (**100**) and *trans*-isoelemicin (**101**) were obtained in addition to **97** from the dichloromethane extract of *Diplolophium buchanani* (Umbelliferae) leaves. These compounds exhibited larvicidal activity against larvae *Ae. aegypti*, with 100% mortality at 25.0 and 100ppm, respectively to **97** and **100 + 101** mixture [79].

Compound 1-(3'-methoxypropanoyl)-2,4,5-trimethoxybenzene (**102**), isolated from the dichloromethane extract of *Cordia alliodora* (Boraginaceae) root bark, exhibited the minimal concentration required to kill all the second instar larvae within 24h at the value of 12.5μg/mL, while β-asarone (**103**) exhibited lethal concentration at 25μg/mL [80]. Moreover α-asarone (**104**) and methyleugenol (**105**) obtained from *A. heterotropoides* (Aristolochiaceae) exhibited LC_{50} values of 26.99 and 57.65ppm, respectively against third instar larvae [75].

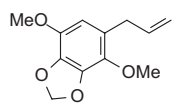
During investigation of the larvicidal activity on red oil of *Myroxylon balsamum* (Fabaceae) three commercial phenylpropanoids were assayed against third instar larvae: cinnamic aldehyde (**106**), eugenol (**107**), and safrole (**108**) exhibited LC_{50} values of 24.4, 44.5, and 49.0ppm, and LC_{90} values of 54.7, 100, and 63.8ppm, respectively [81].

Santos *et al*. have described the larvicidal activity against third instar larvae of *Ae. aegypti* of phenylpropanoids and phenol derivatives generally found in plants [83–89]. Among the tested compounds the LC_{50} values obtained were: 88ppm to eugenol (**107**), 513ppm to vanillin (**109**), 136ppm to salicylaldehyde (**110**), 194ppm to phenol (**111**), 243ppm to (**112**), 577ppm to (**113**), and 177ppm to (**114**) [82]. These compounds have phenol as a common template which showed an LC_{50} of 194ppm. Moreover, the presence of an additional hydroxyl group into aromatic ring has resulted in less potent compounds, while introduction of a methoxyl (**114**) or an aldehyde group (**110**) has resulted in a significant increase of potency. For Santos *et al*., a possible explanation for this structure–toxicity relationship may be the presence of the hydroxyl groups would prevent the substance penetration in the larvae cuticle and reaching its targets [82]. Moreover, piperonal (**115**) presented toxicity against fourth instar larvae of *Ae. aegypti* at 200ppm [90].

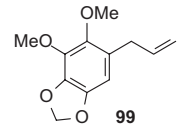
Sodium anacardate (*O*-penta-decadienyl-salicylic acid) (**116**), obtained by extraction of cashew nut shell liquid (CNSL) from *Anacardium occidentale* (Anacardiaceae), had toxic effects against eggs (EC_{50} 162.93ppm), third instar larvae (LC_{50} 55.47ppm), and pupae (LC_{50} 369.78ppm) of *Ae. aegypti* [91].



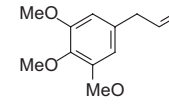
97



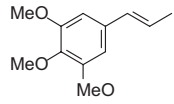
98



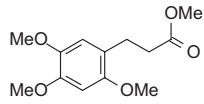
99



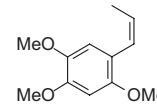
100



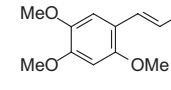
101



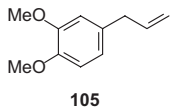
102



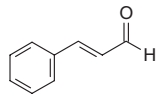
103



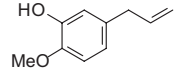
104



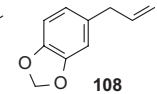
105



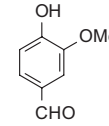
106



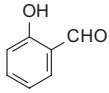
107



108

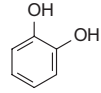


109

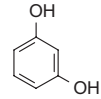


110

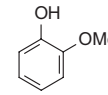
111



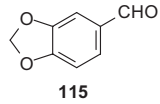
112



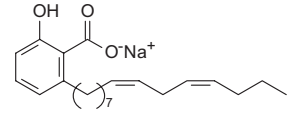
113



114



115



116

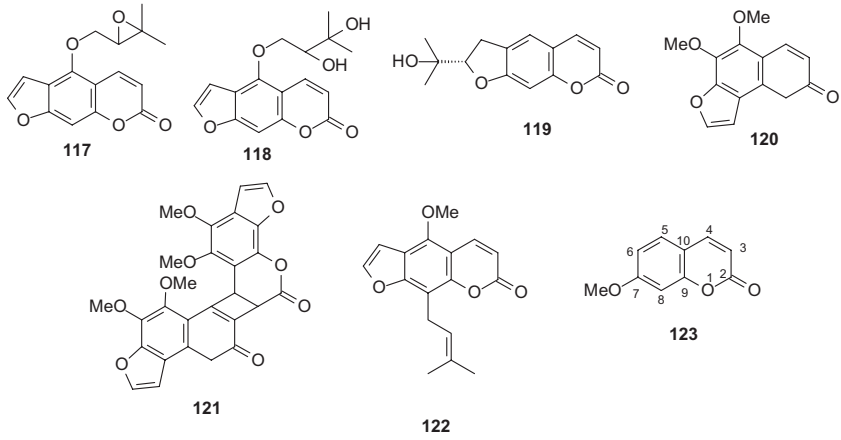
Coumarins

Two furanocoumarins oxypeucedanin (**117**) and oxypeucedanin hydrate (**118**) were isolated through bioassay-guided fractionation of *D. buchanani* (Umbelliferae) leaves and only **117** showed toxicity to *Aedes* mosquito with LC_{100} of 25ppm [79], suggesting therefore, that introduction of a polar group could decrease or lose larvicide action. Marmesin (**119**) was found in roots of *Poncirus trifoliate* (Rutaceae) and showed to be toxic to fourth instar mosquito larvae with LC_{50} of 0.082ppm and LC_{90} of 0.152ppm after 24h [92].

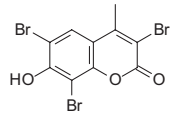
A mixture containing pimpinellin (**120**) and its photodimerization product (**121**), as well as a mixture containing **120** together to swietenocoumarin B (**122**) exhibited LC_{50} of 45.77 and 62.23ppm against fourth instar, respectively [93].

These compounds, identified on the basis of spectroscopic methods, were obtained in addition to other non-larvicidal coumarins from the hexane fraction from the roots of *Esenbeckia grandiflora* (Rutaceae) [93].

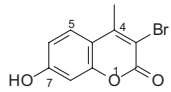
The bioactive hexane extract of *Tagetes lucida* (Asteraceae) against third instar larvae *Ae. aegypti* (LD_{50} 55.7ppm; LD_{90} 107.5ppm) was analyzed by TLC, preparative TLC and GC/MS, from which the 7-methoxy coumarin (herniarine) (**123**) was identified in the bioactive fraction that led 100% mortality at 45ppm [94].



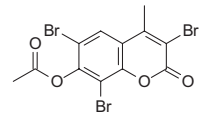
As coumarins have been known for their various biological activities including pesticides and anticoagulants effects, some research groups have prepared some derived compounds and tested them against early fourth instar of *Aedes* mosquito. Deshumukh *et al.* synthesized four 4-methyl-7-hydroxy coumarins **124**–**127**, being **124** the most active with LC_{50} of 2.23ppm [95]. Compounds **125**–**127** had LC_{50} of 34.04, 77.99, and 89.60ppm, respectively, and the coumarin **P124** also decreased hatching of *Ae. aegypti* eggs in 52.5% at 25ppm and caused 100% post-embryonic mortality of first and second instar at 25ppm [95]. Jung and Moon investigated the synthesis of 4-hydroxycoumarin derivatives and produced eight coumarin derivatives (**128**–**135**) [96]. Compounds brofifacoum (**130**), difethialone (**131**), *cis*-flocoumafen (**134**), and *trans*-flocoumafen (**135**) were toxic showing LC_{50}/LC_{90} of 8.23/13.23, 41.35/54.47, 9.34/15.32, and 54.13/74.28ppm, respectively. The other had LC_{50} values greater than 190ppm [96].



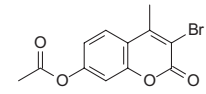
124



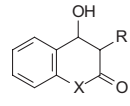
125



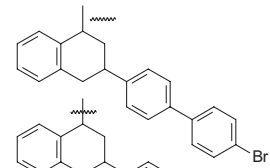
126



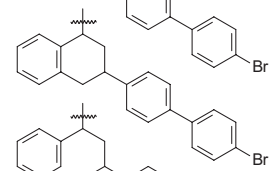
127



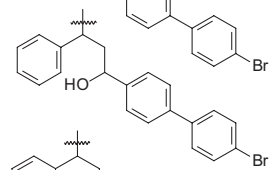
130 X = O, R =



131 X = S, R =

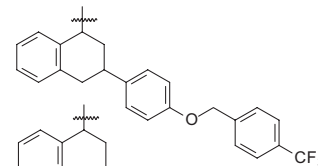


132 X = O, R =

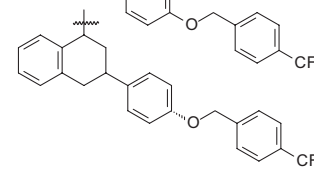


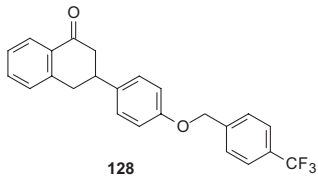
133 X = O, R =

134 X = O, R =

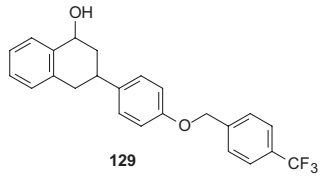


135 X = O, R =





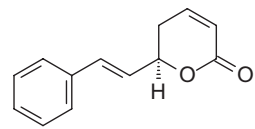
128



129

Styrylpyrones

Goniothalamim (**136**) is a styryldihydropyrone isolated from the bark of *Goniothalamus andersonii* Sinclair (Annonaceae) and from other species of this genus [97], that showed an LC_{50} of 15.0µg/mL and LC_{90} of 57.7µg/mL) against third instar larvae *Ae. aegypti* [46].



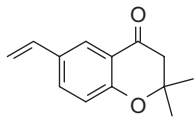
136

Chromanones

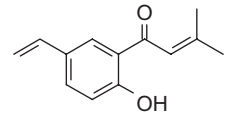
During the studies on chemical composition of plants from the genus *Eupatorium* (Asteraceae), the essential oil from roots of *Eupatorium betonicaeforme* (D.C.) Baker showed LD_{100} of 50ppm against third instar mosquito larvae [98]. Purification

steps were performed resulting in the isolation and identification of two major compounds: 2,2-dimethyl-6-vinylchroman-4-one (**137**) and 2-senecioyl-4-vinylphenol (**138**) that exhibited LD_{100} at 50 and 100ppm, respectively. Moreover, **137** caused 84% mortality at 12.5ppm indicating that it is probably the major active principle responsible for the larvicidal action of the essential oil [98].

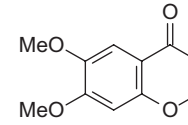
A rare natural chromanone 6,7-dimethoxy-4-chromanone (**139**) was isolated from the seeds of *Derris trifoliate* (Fabaceae) and shown to be toxic to the second instar larvae with LC_{50} of 14.8ppm [99].



137



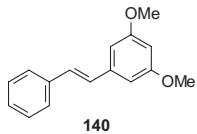
138



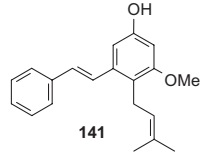
139

Flavonoids

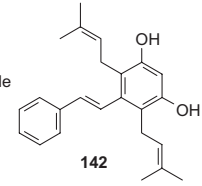
Investigation of the larvicidal extract of *Lonchocarpus chirinanus* (Leguminosae) root bark resulted in the isolation and identification through spectrometric methods, of the stilbene 3,5-dimethoxystilbene (**140**) and seven prenylated stilbenes, named longistylines C–D (**141** and **142**) and chiricanines A–E (**143**–**147**) [100]. Considering structure–activity relationship the nonprenylated stilbene **140** was the compound that exhibited the highest activity with minimal concentration of 3ppm required to kill all the larvae in 24h. Following, **142** and **143** caused 100% mortality at 6ppm, suggesting therefore, that the introduction of prenyl group decreased the toxic effect. Moreover, prenylated stilbenes having a methoxyl group rather than hydroxyl group such as **141** and **145** or cyclised prenyl units as in **144** and **146** were inactive against *Ae. aegypti* larvae. Compound **147** was not tested [100].



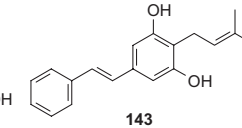
140



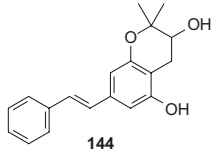
141



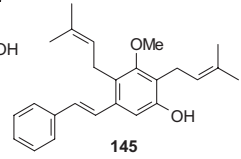
142



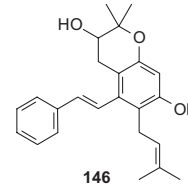
143



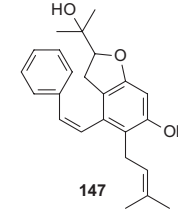
144



145



146

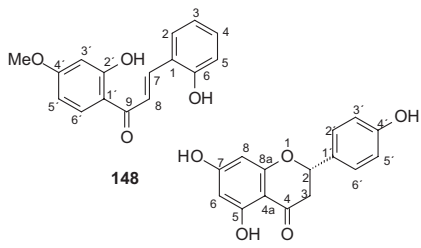


147

The compound 2'-6-dihydroxy-4'-methoxychalcone (**148**) obtained from *Polygonium senegalensis* (Polygonaceae) was toxic to second instar *Ae. aegypti* with LC_{50} of 16.15µg/mL after 48h [101].

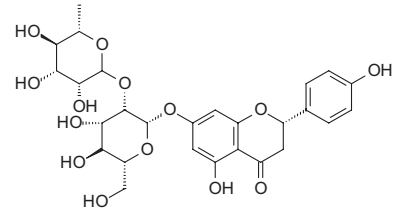
Naringenin (**149**) is the well-known flavanone that exhibited toxicity against late third instar mosquito larvae with LC_{50} of 3.7µg/mL and LC_{90} of 15.1µg/mL [46]. Its glycosylated form named naringin (or naringoside) (**150**) as well as poncirin (**151**), isolated from leaves of *Poncirus trifoliata* (Rutaceae) also showed larvicidal activity against fourth instar stage with LC_{50} of 0.122 and 0.103ppm, and with LC_{90} of 0.223 and 0.191ppm, respectively [92]. Besides, **150**, **151** and the dihydrofuranocoumarin marmesin (**119**) also possessed ovicidal activity and **151** exhibited 100% repellence against this mosquito [92].

Lupinifolin 4'-methyl-ether (**152**) and lupinifolin (**153**) were isolated from the seed pods of *D. trifoliate* (Leguminosae) to be tested against second instar larval stage of *Aedes* and both had an LC_{50} value higher than 20ppm at 24h [102].

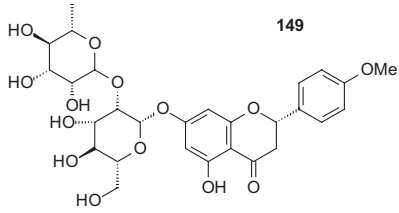


148

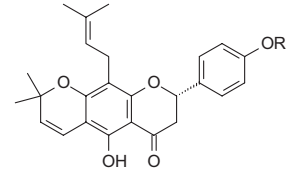
149



150



151



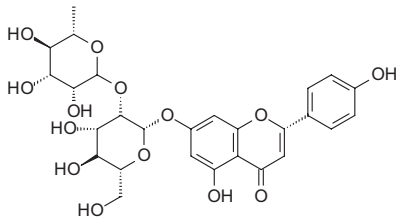
152 R = Me
153 R = H

The glycosylated flavone rhoifolin (**154**), found in the leaves of *P. trifoliata* (Rutaceae), exhibited a toxic effect against fourth instar larvae with LC_{50} and LC_{90} of 0.085 and 0.171ppm, respectively; it also showed 100% repellence against the mosquito and strong oviposition deterrent effect with production of a 66.3% repellence at 2.0ppm [92].

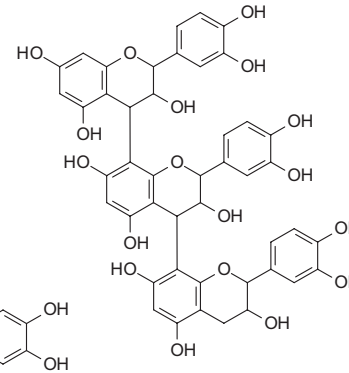
Fractionation of the methanolic extract of *Melicope subunifoliolata* (Rutaceae) leaves was performed to obtain pure bioactive compound, named

meliternatin (**155**) which in turn exhibited larvicidal activity (LC_{50}=0.47μg/mL; LC_{90}=1.45μg/mL) against third instar larvae *Ae. aegypti* [46]. Quercetin (**156**) obtained from *P. senegalensis* (Polygonaceae) was toxic to second instar *Ae. aegypti* with LC_{50} of 16.15μg/mL after 48h [101].

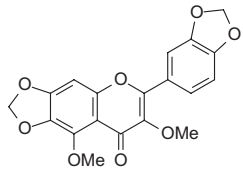
A fraction enriched with the catequic tannin (**157**) obtained from *Magonia pubescens* (Sapindaceae) exhibited a larvicidal action against third instar *Aedes* larvae with LC_{50} of 3.1ppm and LC_{90} of 36.6ppm [103]. Studies to elucidate its mode of action revealed that tannins caused morphological and histological alterations such as extrusion of the peritrophic matrix, that protects the midgut epithelium from chemicals, toxins, pathogens and mechanical damages, vacuolation and absence of cytoplasmatic limits, vesicular apical formation with release of the cytoplasmic contents, increase in the intercellular spaces and detachment of the cells from the basal membrane [104].



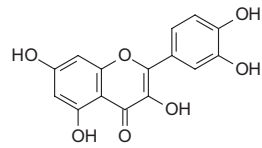
154



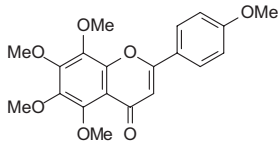
157



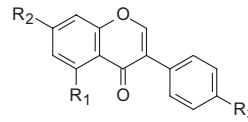
155



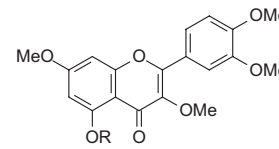
156



158



159 $R_1 = R_2 = R_3 = OH$
160 $R_1 = H$, $R_2 = R_3 = OH$
161 $R_1 = H$, $R_2 = OH$, $R_3 = OMe$
163 $R_1 = R_2 = R_3 = OAc$
164 $R_1 = H$, $R_2 = R_3 = OAc$
165 $R_1 = H$, $R_2 = OAc$, $R_3 = OMe$



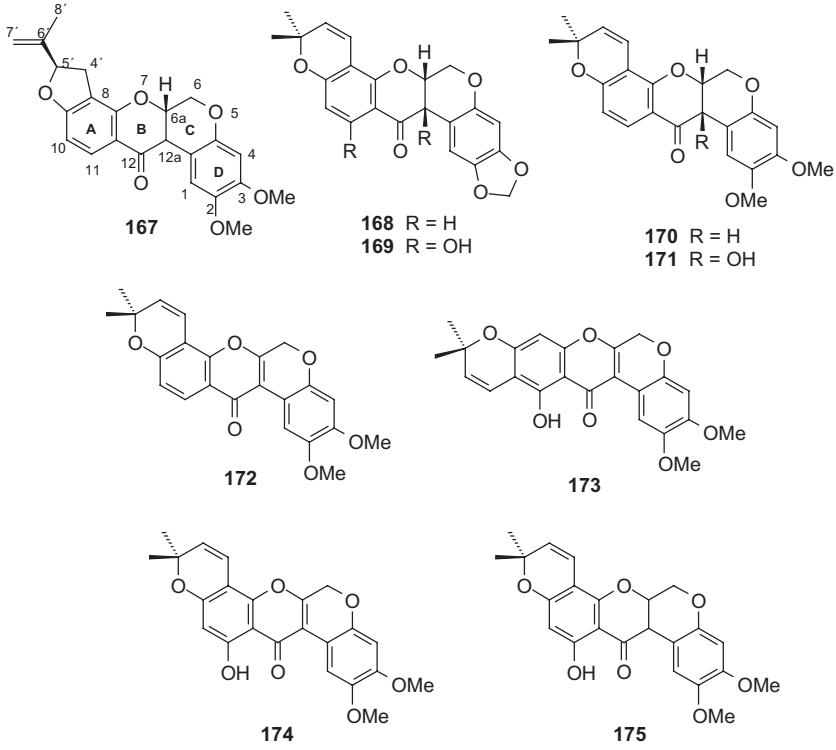
162 R = H
166 R = Ac

From the culture broth of the filamentous bacteria *Streptomyces* sp. (**85–88**) cultivated on soybean were isolated three flavonoids: the flavone tangeterin (**158**) and the isoflavonoids genistein (**159**) and daidzein (**160**) that arise from hydrolysis of the corresponding glycosides present in soybean by glycosidases produced by this micro-organism [105]. Compounds **159** and **160**, in addition to flavonoids formononetin (**161**) [106] and retusin (**162**) [107] were acetylated producing their respective acetylated forms (**163–166**). All compounds were assayed against third instar larval of *Aedes* mosquito and their LC_{50} values were calculated after 48h. The acetate derivatives **163**, **164** and **165** were more toxic (with LC_{50} value of 4.0, 2.0, and 2.5ppm, respectively) than their corresponding flavonoids **159**, **160**, and **161** that exhibited LC_{50} value of 10.8, 7.6, and 10.7ppm, respectively. Isoflavone **158** showed LC_{50} of 7.2ppm and retusin (**162**) and its acetylated derivative (**166**) was not active [105].

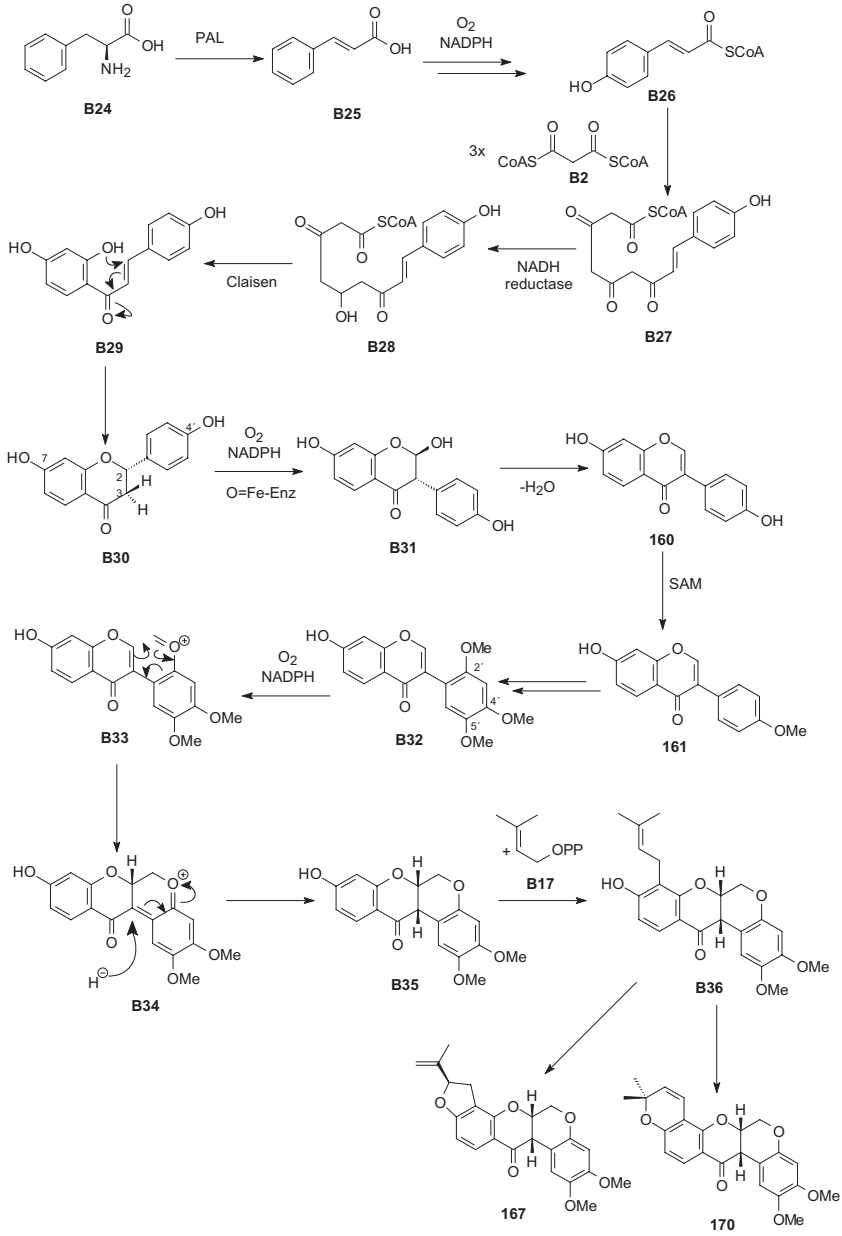
Rotenone (**167**) has been reported in 12 genera of plants belonging to Leguminosae, containing the genus *Tephrosia* the largest number of rotenoid producing species [108]. This compound caused 100% mortality within 3 days of fourth instar larvae of *Ae. aegypti* at a concentration of 10ppm [109]. Crude methanol extract of the seeds from *Derris foliate* exhibited LC_{50} of 0.74μg/mL against second instar of *Aedes* and its fractionation revealed that **167** is the major compound in this extract, being responsible for the toxicity against this mosquito with LC_{50} of 0.47μg/mL [99].

Other rotenoids were obtained from the larvicidal chloroform extract of seeds from *Millettia dura* Dunn (Leguminosae) (LC_{50} value of 3.5ppm at 24h against second instar larvae stage) [110]. They were isolated previously from *M. dura* in addition to the **167** and were identified as millettone (**168**), millettosin (**169**), deguelin (**170**), and tephrosin (**171**) [111]. Compounds **168**–**171** and dehydrodeguelin (**172**) (prepared by dehydration of **171**) were evaluated against second instar [110]. LC_{50} values of 1.6 and 1.4ppm were registered for **170** and **171**, respectively. Rotenoids **168** and **169** were not active up to 20μg/mL, suggesting the importance of methoxyl groups at C-2 and C-3 for the toxic effects. Moreover, **172** was inactive, which can be attributed to the presence of a double bond between B/C ring junction [110]. Besides, compounds **170** and **171** also caused 100% and 95% mortality of fourth instar larval stage, respectively, at a concentration of 5μg/mL [109].

Fractionation of bioactive roots extract of *Tephrosia toxicaria* Pers (LC_{50} 47.86ppm) resulted in the isolation and identification of three known rotenoids namely 6a, 12a-dehydroβ-toxicarol (**173**), 6a, 12a-dehydroα-toxicarol (**174**), and α-toxicarol (**175**), but only **175** showed larvicidal activity against third instar with LC_{50} 24.55ppm. Compound **173** was not tested and **174** had an LC_{50} superior to 50ppm [112].



Biosynthetic work on the rotenoids are summarized in the review written by Crombie and Whiting [113]. These compounds are characterized as isoflavonoids according to the biosynthetic studies. Conversion of *L*-phenylalanine (**B24**) into trans-(*E*)-cinnamic acid (**B25**) through elimination of ammonia from the side chain *via* the enzyme *L*-phenylalanine ammonia lyase (PAL) with subsequent hydroxylation by cinnamate 4-hydroxylase and thioesterification *via* 4-coumarate Coa ligase give 4-hydroxycinnamoyl-CoA (**B26**) as shown in Scheme 5. **B26** is the starter unit that condenses with three molecules of malonyl-CoA (**B2**) producing the intermediate **B27** that lose one of the hydroxyl groups due to the action of a reductase enzyme (**B28**) and the isoliquiritigenin (**B29**) is formed *via* chalcone synthase intervention. A Michael-type nucleophilic attack of hydroxyl on to α,β-unsaturated ketone at **B29** produces the flavanone liquiritigenin (**B30**). Afterwards, a rearrangement with 1,2-aryl group migration takes place initiated by isoflavone synthase, an NADPH dependent P450-iron-containing enzyme, transforms **B30** into the isoflavone daidzein (**160**) *via* intermediate hydroxyisoflavanone (**B31**).



SCHEME 5 Proposed biosynthetic pathway to rotenone (**167**) and deguelin (**170**). Adapted from [113] and [10].

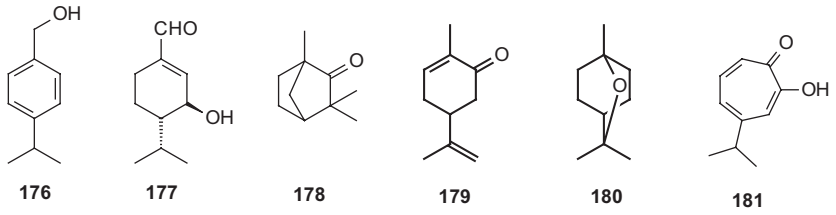
A methoxyl group is inserted in **161** by *S*-adenosylmethionine (SAM) to produce formononetin (**161**) followed by hydroxylation/methoxylation phase producing the intermediate 7-hydroxy-2',4',5'-trimethoxy isoflavone (**B32**). Oxidation of 2'-methoxy group at **B32** (hydroxylation and loss of hydroxide) would give **B33** and electrocyclization (**B34**) and hydride donation produces demethylmunduserone (**B35**). Next, introduction of a C5 isoprene unit (**B17**) *via* dimethylallylation of **B35** (C-alkylation at activated position ortho to phenol) gives rotenonic acid (**B36**) which under cyclization to five-membered ring produces rotenone (**167**) or to six-membered that forms the dimethylpyrane system of deguelin (**170**) [10,113].

Rotenone (**167**) and other rotenoids are powerful insecticides since they interfere with oxidative phosphorylation, blocking transfer of electrons to ubiquinone by complexing with NADH:ubiquinone oxidoreductase of the respiratory electron transport chain, being rapidly inactivated in the presence of light and air [10].

Terpenoids

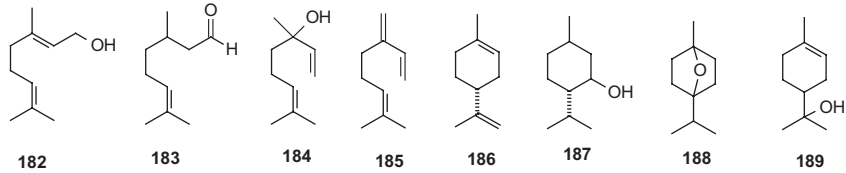
Bioassay-guided chromatography of essential oil of *Eucalyptus camadulensis* (Myrtaceae) resulted in the isolation and characterization (by NMR analysis and synthesis) of 4-isopropylbenzyl alcohol (**176**) and eucamalol (**177**) [114]. These compounds exhibited potent mosquito-repelling activities against *Ae. aegypti* immediately after their applications (100% repellency), and 1h after treatment, **176** and **177** exhibited 23% and 92% repellency, respectively compared with the commercial DEET that showed 84% activity. Among this, **177** showed 75% repellency even 3h after application [114]. Moreover, (+)-fenchone (**178**), isolated from the methanol extract of fruits from *Foeniculum vulgare* [Apiaceae (Umbelliferae)], exhibited repellent activity against hungry *Ae. aegypti* females with 94% and 82% repellency at 0.01 and 0.005mg/cm^2, respectively and at a dosage of 0.4mg/cm^2; **178** exhibited 76% repellent activity at 30min after treatment [41].

The major insecticidal principle obtained from dill greens (*A. graveolus* L., Umbelliferae) was the monoterpenoid *d*-carvone (**179**) that showed toxicity against third instar of *Aedes* larvae with LC_{50} value of 107ppm [78]. The essential oil from leaves of *Hyptis martiusii* Benth (Labiatae) was tested against third instar larvae of *Ae. aegypti* inducing 100% mortality after 24h at a dosage of 250ppm [115]. The oxygenated monoterpene 1,8-cineole (**180**), isolated by chromatographic procedures, was the major constituent (24.27%) and produced 100% mortality at 100ppm [115]. Moreover, bioassay-monitored fractionating of hexane fraction of the methanol extract from leaves of *Chamaecyparis obtuse* (Cupressaceae) resulted in the isolation of β-thujaplicin (**181**) that showed larvicidal activity (LC_{50} = 2.91ppm) against fourth instar larvae of *Ae. aegypti* [116].

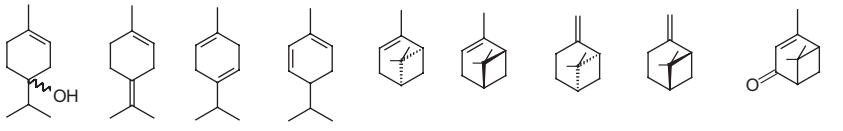


176 177 178 179 180 181

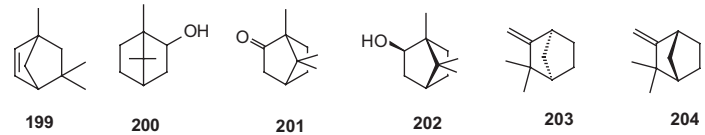
Many plant essential oils have been reported to possess larvicidal activities against *Aedes* mosquito. Their chemical compositions are usually determined by spectroscopic methods such as gas-chromatography–mass spectrometry (GC/MS) and the identification of these compounds is confirmed by comparison of their GC retention times with authentic compounds (purchase from Chemical Companies) or mass spectral libraries. Furthermore, these chemicals (**182–213**) were also evaluated against third instar larval stage in order to verify the bioactive components of essential oils. Table 1 shows the larvicidal activities (LC_{50}) of evaluated monoterpenoids on *Aedes* larvae. It could be observed that some of the present LC_{50} values differ from reported data which may be the result of different methodologies, differences on mosquito susceptibilities or purity of the chemicals.



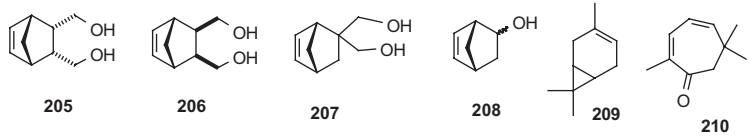
182 183 184 185 186 187 188 189



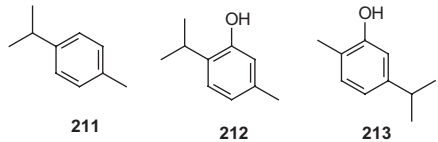
190 191 192 193 194 195 196 197 198



199 200 201 202 203 204



205 206 207 208 209 210



211 212 213

Due to different LC_{50} values found in the literature for monoterpenoids it becomes difficult to propose the structure–toxicity relationships between them. However, some evidences could be commented. For example, removal of the hydroxyl group from thymol (**212**) or carvacrol (**213**) increased larvicide potency as observed for *p*-cymene (**211**) [82], and differences on LC_{50} values from enantiomeric (+)- and (−)- monoterpenoids (**194–197**, **203–204**), suggesting a chiral recognition by receptors and enzymes [82]. The position of the double bond may alter the activity as observed for α- and β- pinenes (**194–197**) [81].

TABLE 1 Larvicidal Activities (LC_{50}) of Evaluated Monoterpenoids on Third Instar Larvae of *Ae. aegypti*

Compound	LC_{50}, ppm	Ref.	Compound	LC_{50}, ppm	Ref.
geraniol (**182**)	81.6	[81]	verbenone (**198**)	93.16	[117]
citronelal (**183**)	>100	[81]	fenchene (**199**)	69.28	[81, 117]
linalool (**184**)	>100[a], 96.6[b]	[81][a], [117][b]	borneol (**200**)	94.89[a] 610[b]	[117[a], 82[b]]
myrcene (**185**)	66.42	[117]	(+)-camphor (**201**)	657	[82]
(+)-limonene (**186**)	24.47	[117]	isoborneol (**202**)	598	[82]
d-carvone (**179**)	107[a], 43.8[b]	[78][a], [81][b]	(+)-camphene (**203**)	406[a] 67.02[b]	[82][a], [117][b]
(−)-menthol (**187**)	>100	[81]	(−)-camphene (**204**) 84.6%	220	[82]
1,8-cineole (**180**)	74.91[a], 1419[b], 57.2[c]	[117][a], [82][b], [118][c]	5-norbonene-2-endo,3-endo-dimethanol (**205**)	1407	[82]
1,4-cineole (**188**)	751	[82]	5-norbonene-2-exo,3-exo-dimethanol (**206**)	717	[82]
α-terpineol (**189**)	111.78	[117]	5-norbonene-2, 2-dimethanol (**207**)	785	[82]
terpine-4-ol (**190**)	64.76	[117]	5-norbonene-2-ol (**208**)	759	[82]
terpinolene (**191**)	15.32	[117]	Δ^3-carene (**209**)	19.19	[117]

Continued

TABLE 1 Larvicidal Activities (LC_{50}) of Evaluated Monoterpenoids on Third Instar Larvae of *Ae. aegypti*—Cont'd

Compound	LC_{50}, ppm	Ref.	Compound	LC_{50}, ppm	Ref.
γ-terpinene (**192**)	17.11	[117]	eucarvone (**210**)	130.35	[117]
α-phellandrene (**193**)	23.08	[117]	β-thujaplicin (**181**)	2.91	[116]
(+)-α-pinene (**194**)	74.3[a], 50.92[b], 15.4[c]	[81][a], [117][b], [118][c]	*p*-cymene (**211**)	51	[82]
(−)-α-pinene (**195**)	64.8	[117]	thymol (**212**)	81	[82]
(+)-β-pinene (**196**)	42.5[a], 22.39[b], 12.1[c]	[81][a], [117][b], [118][c]	carvacrol (**213**)	69	[82]
(−)-β-pinene (**197**)	15.40	[117]			

The sesquiterpene *E*-nerolidol (**214**) was isolated from the bioactive hexane fraction (LC_{50} 13.0ppm) of ethanolic extract from *M. balsamum* (Fabaceae) red oil. **214** caused 50% mortality of third instar larvae of *Aedes* at a concentration of 17.0ppm, being less active than its original fraction [81]. This may suggest a synergism effect into this fraction with minoritary components. Besides, its isomeric form, *E,E*-farnesol (**215**) exhibited an LC_{50} value of 13.0ppm [81].

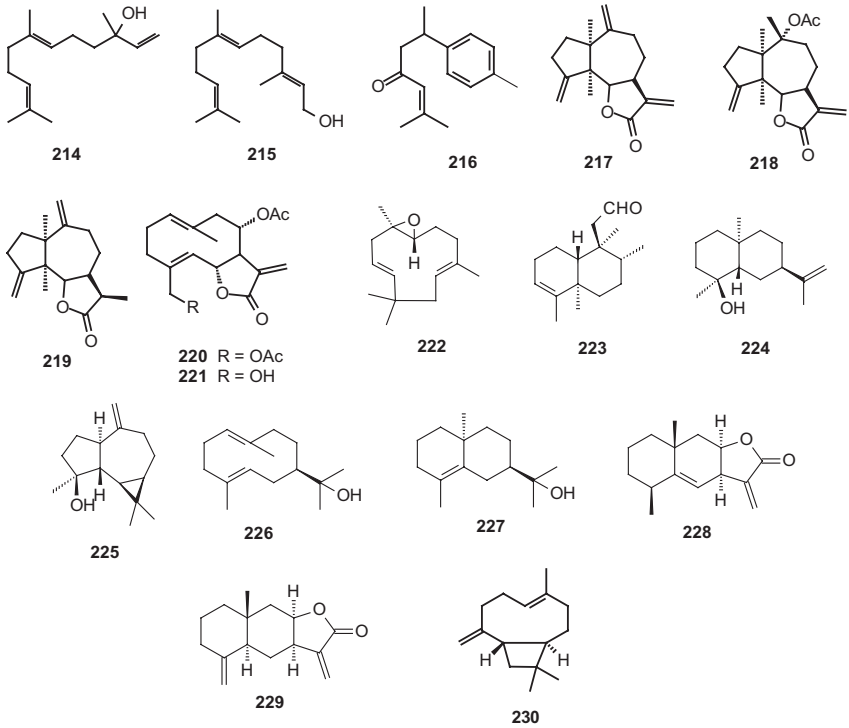
ar-Turmerone was obtained from volatile oil of rhizomes of *Curcuma longa* (Zingiberaceae) (**216**) that caused 100% mortality at 50ppm on fourth instar *Aedes* mosquito [119]. Fractionation of the dichloromethane extract of *Targionia lorbeeriana* (Targionaceae) afforded three guaianolide-type sesquiterpene lactones and two germacranolides [120]. Their structures were assigned by spectroscopic methods (NMR and MS) as dehydrocostus lactone (**217**), acetyltriflocusolide lactone (**218**), 11-α-H-dihydrodehydrocostus lactone (**219**) acetylsalonitenolide (**220**), and 8-acetylsalonitenolide (**221**). Larvicidal assay against larvae of *Aedes* were performed and LD_{100} of 12.5, 50, and 50 were registered, respectively, to **217**, **218**, and **219**, while compounds **220** and **221** did not caused death of larvae [120].

Two Verbenaceae plants, *Callicarpa americana* and *C. japonica*, were examined for their bite-deterring constituents and, bioassay-guided fractionation using *Ae. aegypti* mosquitoes, afforded three bioactive compounds from both plants, namely humulene epoxide II (**222**), callicarpenal (**223**),

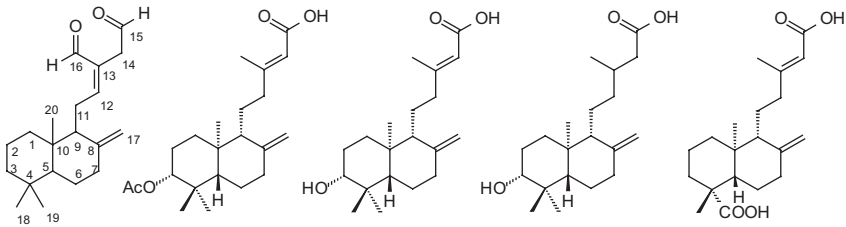
and intermedeol (**224**), as well as spathulenol (**225**) from *C. japonica* [121]. These sesquiterpenes, which showed significant deterrent effect, had their structures assigned by means of spectroscopic methods and comparison of data reported in the literature [121]. In addition, callicarpenal (**223**), when applied to cloth at 25nmol/cm^2, deterred the biting of *Aedes* mosquito more effectively than DEET (proportion of mosquitoes not biting of 0.91 and 0.67 for **223** and DEET, respectively; control treatment was 0.21) [87]. Moreover, a selection of 12 sesquiterpenes were evaluated for their repellent activity, but only two hedycaryol (**226**) and 10-*epi*-γ-eudesmol (**227**) showed the highest levels of repellency with 100% and 82.2% repellency after 60min, respectively, at a concentration of 78.6μg/cm^2, while DEET caused 71.9% repellency [122].

Randomly screening for new pesticides (against first instar of *Ae. aegypti*) resulted in the isolation of two eudesmanolides, alantolactone (**228**) and isoalantolactone (**229**) from *Inula helenium* (Asteraceae), that exhibited LC_{50} values of 36.2 and 10.0ppm, respectively [123]. Moreover, several analogues of **228** and **229** were designed but none of the synthetic isomers were more active than **229** itself [123].

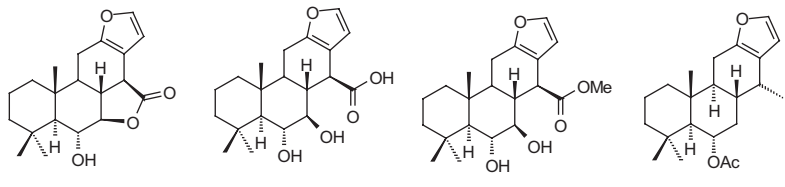
The sesquiterpene *β*-caryophyllene (**230**) also exhibited larvicidal effect against third instar of *Aedes* with LC_{50} value of 88.30ppm [117].



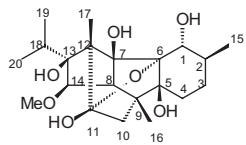
Labdane-diterpenoid labda-8(17),12-diene-15,16 dial (**231**) was isolated through bioassay-guided fractionation of ethyl acetate extract from *C. longa* L. (Zingiberaceae) rhizomes and led to 100% mortality of fourth instar *Aedes* larvae at 10μg/mL within 48h, as well as showed antifungal activity against three different species of Candida [119]. Moreover, studies on larvicidal activity of oil-resin from *Copaifera reticulata* Ducke (Leguminosae) led to the isolation of four *ent*-labdane diterpenoids (−)-3β-acetoxylabdan-8(17)-13-dien-15-oic acid (**232**), *ent*-alepterolic acid (**233**), (−)-3β-hydroxylabdan-8(17)-en-15-oic acid (**234**), and *ent*-agatic acid (**235**) [124]. The toxicity of these compounds against third instar larvae were determined and diterpenoids **232** and **233** exhibited LC_{50} (and LC_{90} values) of 0.8ppm (8.2ppm) and 87.3ppm (128.8ppm), respectively. The two latter diterpenoids were not active against these larvae [124]. Besides, ultrastructural alterations in the midgut such as cytoplasmatic vacuolation, cell disruption and degeneration caused mortality of larvae when treated with **232** after 22h were reported [125].



231 **232** **233** **234** **235**



236 **237** **238** **239**



240

Bioassay-guided fractionation of the ethanolic extract from seeds of *Pterodon polygalaeflorus* (Benth) (Leguminosae) led to the isolation of three

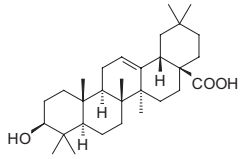
vouacapane diterpenoids 6α-hydroxyvouacapane-7β,7α-lactone (**236**), 6α,7β-dihydroxyvouacapan-17β-oic acid(**237**), and methyl 6α,β-dihydroxyvaoucapan-17β-oate (**238**) that exhibited larvicidal activity against fourth stage larvae of *Ae. aegypti* after 24h [126]. **237** was the most active larvicide with LC_{10}, LC_{50}, and LC_{90} values of 7.85, 14.69 and 27.50μg/mL, respectively, followed by **238** which showed LC_{10}, LC_{50}, and LC_{90} values of 18.85, 21.76, and 25.13μg/mL. Diterpenoid **236** was toxic to larvae with LC_{10}, LC_{50}, and LC_{90} values of 28.73, 50.08, and 87.31μg/mL, respectively [126], indicating that the presence of a γ-lactone caused the reduction of the larvicidal activity. In addition the furane-diterpenoid 6-α-acetoxy vouacapane (**239**), obtained from *P. polygalaeflorus* fruits, showed an LC_{50} of 186.21ppm against third stage larval of *Aedes* mosquito [127].

Ryanodane diterpene isolated from ripe fruit of *Erythroxylum passerinum* (Erythroxylaceae) was identified as 14-*O*-methyl ryanodanol (**240**) by comparison with literature data and tested for its larvicidal activity against first instar with LC_{50} of 82ppm [128].

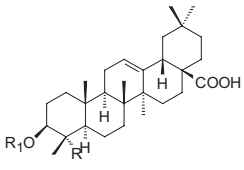
Oleanolic acid (**241**) is a triterpene present in more than 120 plants with diverse biological effects such as antitumoral, antiulcerogenic, hepatoprotector and HIV-1 protease inhibitor [129]. Among this, **241** also presented toxicity against first instar of *Aedes* larvae with LC_{50} of 4.4ppm [130].

A combination of chromatographic and spectroscopic techniques resulted in the isolation and identification of four monodesmoside saponins **242**–**245** and one bidesmoside saponin **246**. All saponins were submitted to assay for their larvicidal activity against third instar larval stage, giving LC_{50} values of 18.6, 25.1, 27.9 and 104.7ppm, respectively, to **242**–**245**. The bidesmoside saponin **246** did not cause any mortality suggesting, therefore, the influence of a free carboxyl group in the larvicidal effect [131]. Saponin **247** is the main saponin found in root-derived callus of *Balanites aegyptiaca* (Balanitaceae) that showed to be a larvicidal dose-dependent with 30, 63, and 88% mortality of third instar at 500, 1000 and 1500ppm after 48h, respectively [132].

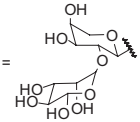
Two tetranortriterpenoids named nimocinolide (**248**) and isonimocinolide (**249**), isolated from leaves of *Azadirachta indica* (Meliaceae), have been reported as mutagenic agent against *Aedes* mosquito [133] and larvicidal toxicity with LC_{50} 0.625 and 0.74ppm, respectively [12]. Continuous studies on this plant have led to the isolation of 23-*O*-methylnimocinolide (**250**) and 7-*O*-deacetal-23-*O*-methyl-7-*O*-senecioylnimocinolide (**251**) that showed toxicity against fourth instar larvae with LC_{50} 53.0 and 2.14ppm, respectively [134]. **251** was 24 times more toxic than **250** and the difference is due to senecioyloxy group in the former instead of acetoxy group in the latter [134]. In addition, 6α-*O*-acetyl-7-deacetylnimocinol (**252**) and nimocinol (**253**) also showed larvicidal properties with LC_{50} values of 21 and 83ppm, respectively [135].



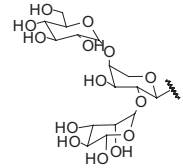
241



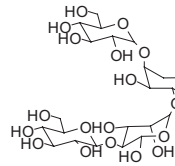
242 R = CH_2OH, R_1 =



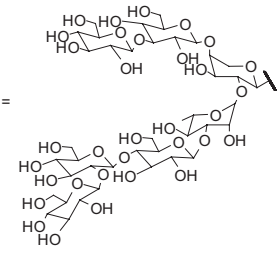
243 R = CH_3, R_1 =

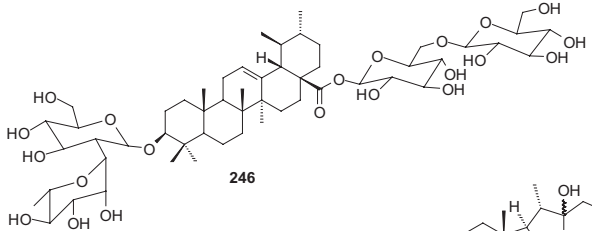


244 R = CH_3, R_1 =



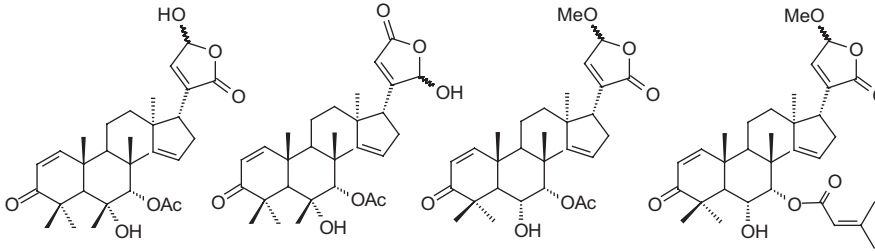
245 R = CH_2OH, R_1 =

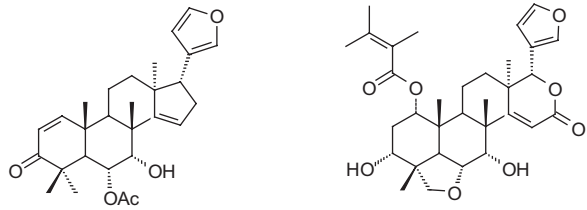




246

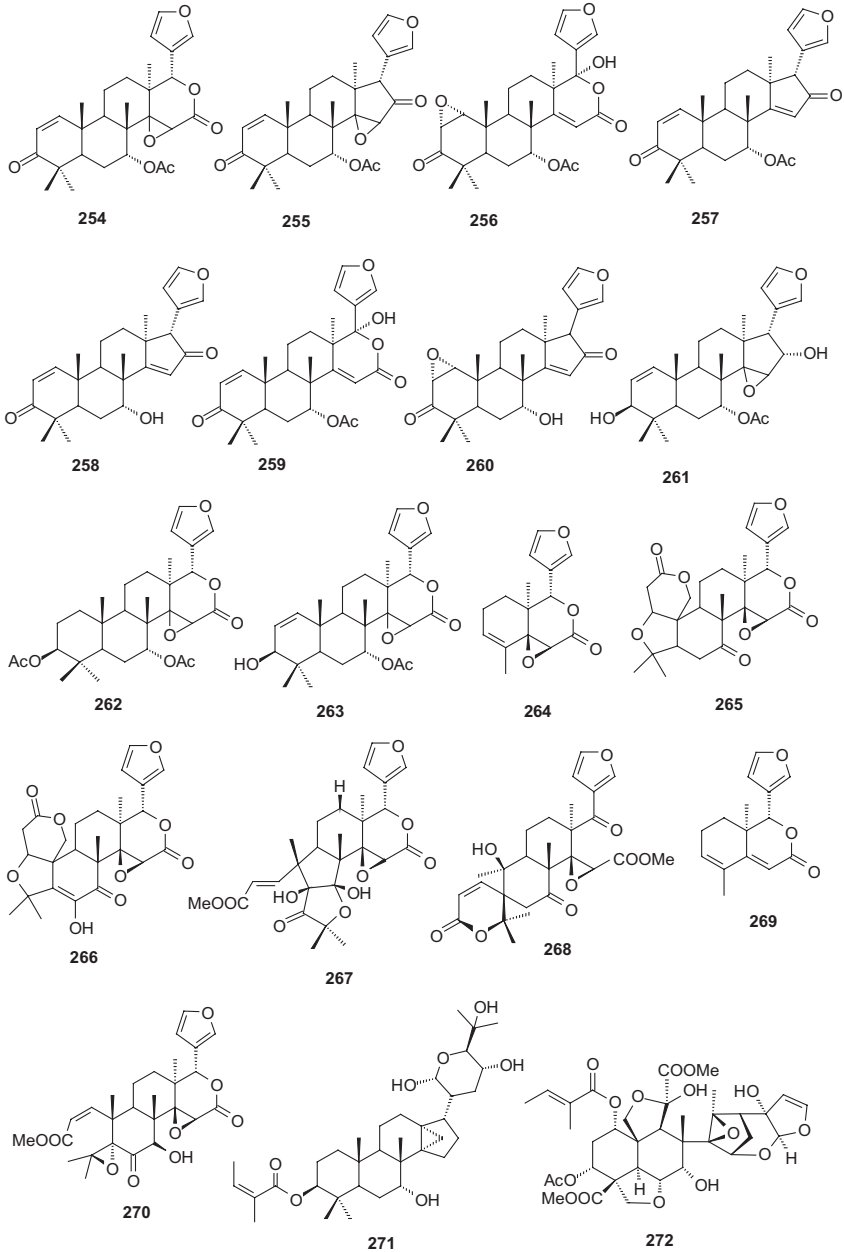
247





Natural and derivatives gedunin-related limonoids were tested against fourth instar larvae of *Ae. aegypti* at 10 and 50ppm [136]. Gedunin (**254**) and epoxyazadiradione (**255**) exhibited 100% toxicity at 50ppm, while epoxynimolicinol (**256**) displayed approximately 50% mortality. Compounds azadiradione (**257**), nimbocinol (**258**), nimolicinol (**259**), and 1α,2α-epoxynimbocinol (**260**) showed toxicity with % mortality between 20 and 40%, while the compounds 3,3-*O*-16,16-*O*-tetrahydroepoxyazadiradione (**261**), diacetate of tetrahydrogedunine (**262**), and 3,3,-*O*-dihydrogedunine (**263**) did not caused any larvae death after treatment [136]. Only **254** have displayed 100% larvicidal effect at 10ppm [136].

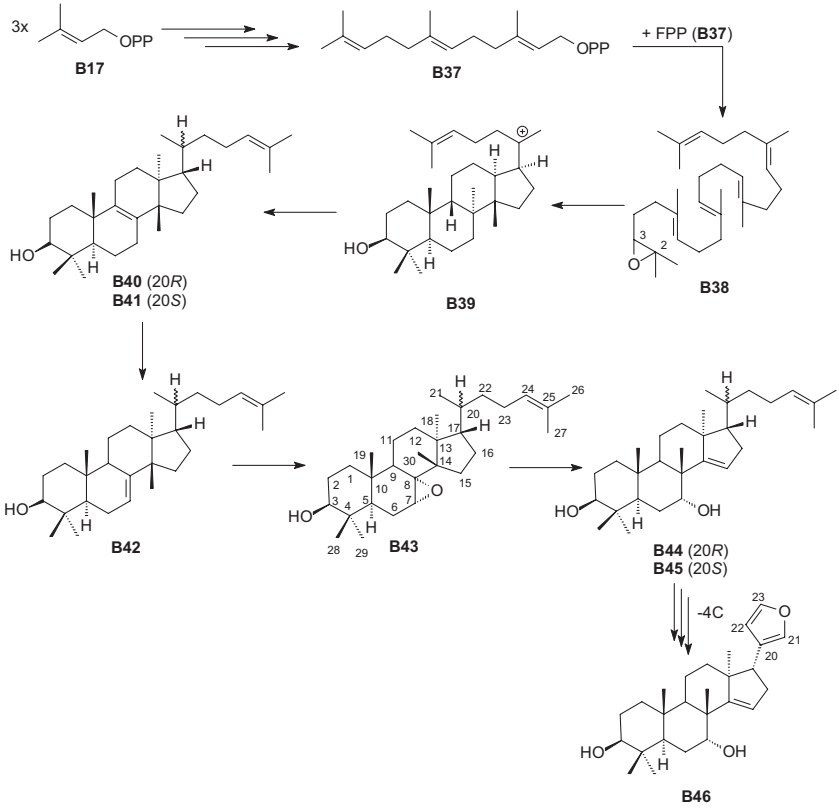
Calodendrolide (**264**), limonin (**265**) and limonin diosphenol (**266**) were isolated from root barks and seeds of *Calodendrum capense* Thumb (Rutaceae) and tested for their larvicidal properties against second instar mosquito larvae and displayed the following LC_{50} values of 13.1, 71.6 and 217.1ppm, respectively [137]. Further three additional limonoids harrisonin (**267**), pedonin (**268**), and pyroangolensolide (**269**) were also obtained and showed LC_{50} values of 16.6, 28.1, and 59.2μM, respectively [138].



From methanol extract of stems from *Spathelia excels* (Rutaceae) were obtained the limonoid deacetylspathelin (**270**) and a glabretal-type triterpenoid (**271**) that showed larvicidal activity against third instar larvae with LC_{50} 69 and 4.8ppm, respectively [139].

Neem formulation of azadirachitin (**272**) (Niconeem, 0.03% of **272**), a known limonoid possessing insecticidal activities, was tested against fourth instar larvae of *Ae. aegypti* and showed an LC_{99} 20.9ppm [140]. Its mode of action is different of other insecticides since **272** did not kill the insects immediately; it attacks the insect's reproductive cycle, its feeding pattern and, therefore, these insects are not able to reproduce, eat or grow [141].

Limonoids are tetranortriterpenoids derived biosynthetically from euphane or tirucallane triterpenoids. Proposed biosynthetic pathway for these compounds starts with carbocation mediated cyclization of squalene 2,3-oxide (**B38**) producing the tertiary protosteryl cation (**B39**) that gives their precursors euphol (20*R*) (**B40**) or tirucallol (20*S*) (**B41**) through a series of concerted Wagner–Meerwein migrations of methyls and hydrides (Scheme 6) [10,12,141]. An allylic isomerisation led to butyrospermol (**B42**), followed by epoxidation to a 7-epoxide (**B43**), and subsequent epoxide opening through Wagner–Meerwein rearrangement of methyl group at C-14 to C-8 give *apo*-euphol (**B44**) and *apo*-tirucallol (**B45**) intermediates. Oxidative degradation at C-17 side chain of



SCHEME 6 Proposed biosynthetic pathway to limonoids [10,12,141].

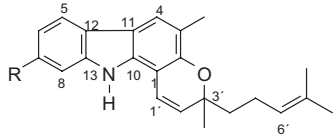
either of these precursors results in loss of four carbon atoms and formation of β-substituted furan (**B46**). Further oxidations and skeletal rearrangements in one or more rings give rise to different groups of limonoids [10,12,141].

Alkaloids and Amides

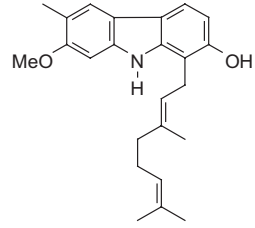
Mosquitocidal, antimicrobial and topoisomerase I and II bioassays guided fractionation from the crude acetone extract from the leaves of *Murraya koenigii* (Rutaceae) resulted in the isolation of the carbazole alkaloids mahanimbine (**273**), murrayanol (**274**) and mahanine (**275**) [142]. Their structures were confirmed by ^{1}H and ^{13}C NMR spectra data and their mosquitocidal properties were evaluated against fourth instar larval *Aedes* with LD_{100} of 100, 12.5 and 12.5ppm, respectively, to **273**, **274**, and **275**. The latter also exhibited 69% mortality at 1ppm [142]. The increase of toxic properties of **274** and **275** could be attributed to the oxidation at C-7 carbon in these compounds.

The bioactive methanol extract from the roots of *Rollinia leptopetala* (Annonaceae) against third instar mosquito larvae (LC_{50} 64.6ppm) was fractionated by column chromatography to give the oxoaporphine alkaloid liriodenine (**276**) that had an LC_{50} of 3.6ppm in addition to its antibacterial and cytotoxic properties [143]. Another piperidine alkaloid named *N*-methyl-6β-methoxy-2β-methylpiperidine (**277**) was obtained from *Microcos paniculata* (Tiliaceae) stem bark and showed LC_{50} of 2.1ppm against second instar larvae stage [144].

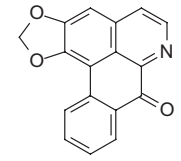
The purine alkaloid caffeine (**278**) is a methyl derivative of xanthine and co-occurs in plants such as *Coffea* species (Rubiaceae), *Thea sinensis* (Theaceae) – tea, *Cola* species (Sterculiaceae), *Theobroma cacao* (Sterculiaceae), *Ilex paraguensis* (Aquifoliaceae), and *Paullinia cupana* (Sapindaceae) [10]. This alkaloid blocked the development the early stages of mosquito *Aedes* with LD_{100} of 1.0mg/mL (1000ppm), probably by affecting esterase enzymes that are involved in several physiological processes, including insecticide degradation [145].



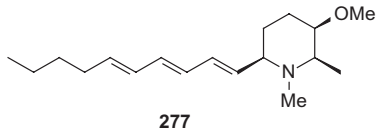
273 R = H
275 R = OH



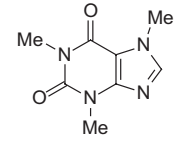
274



276



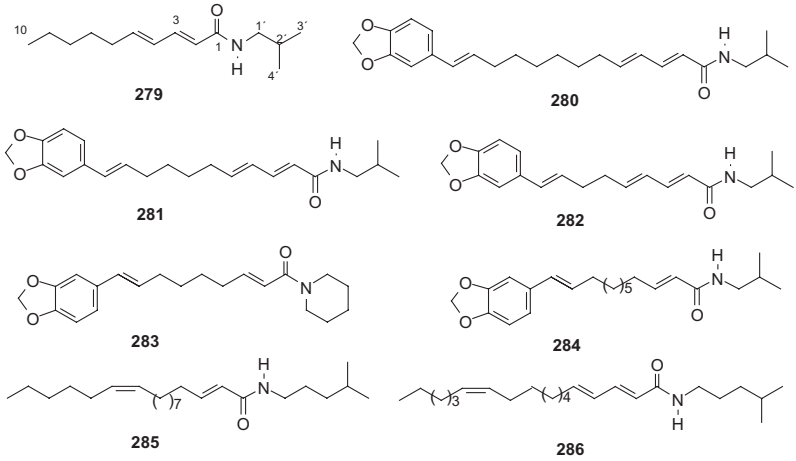
277

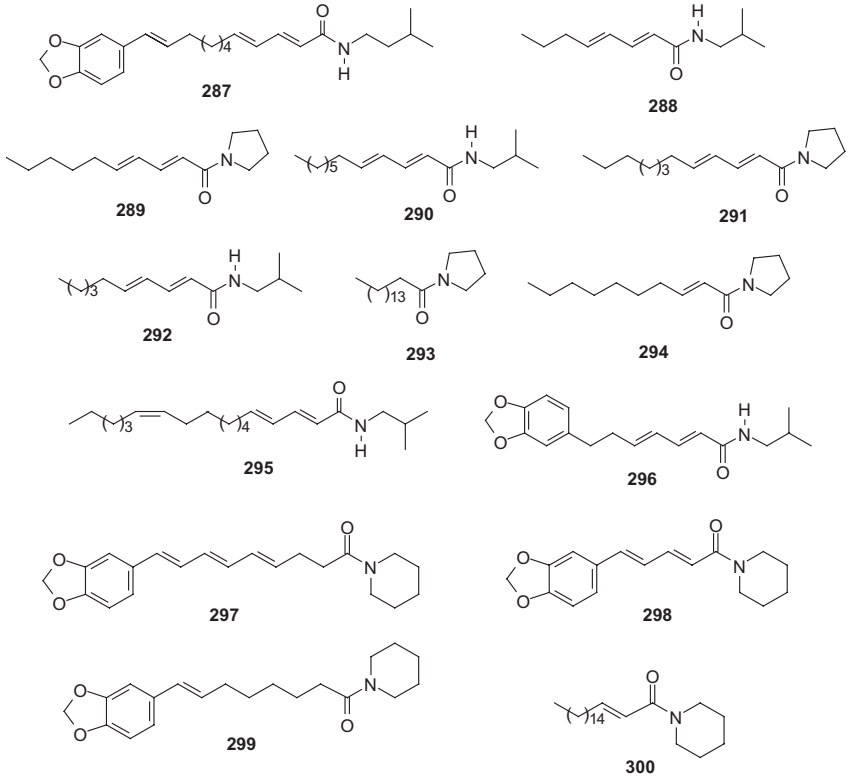


278

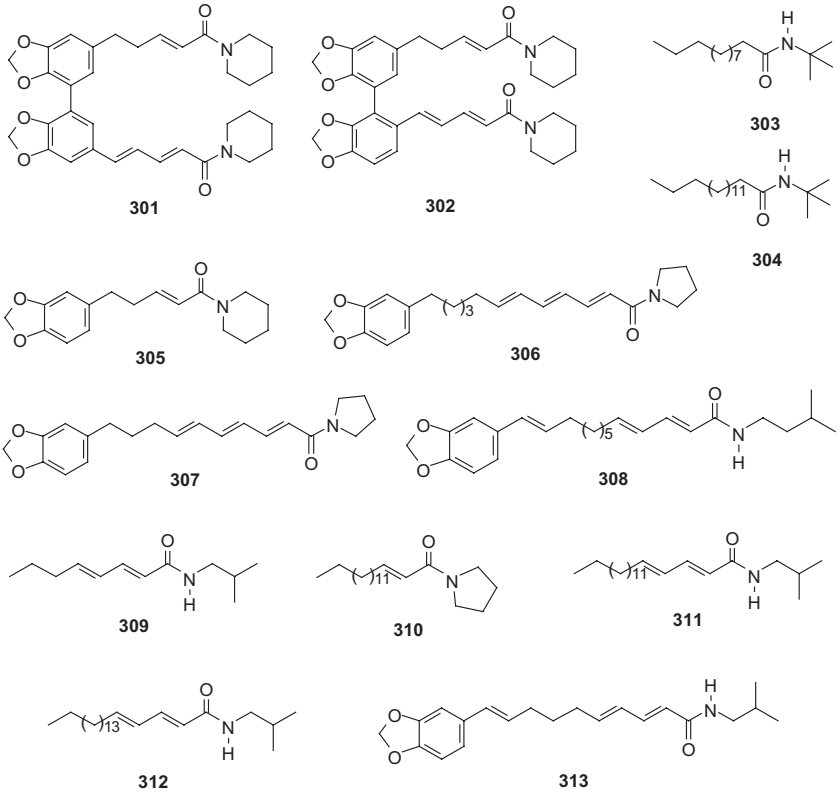
Insecticidal bioassay-guided fractionation of the *Piper nigrum* (Piperaceae) fruit extract afforded four *N*-isobutylamine alkaloids identified by spectroscopic analyses as pellitorine (**279**), guineesine (**280**), pipercide (**281**), and retrofractamide A (**282**) [146]. Compounds **281** and **282** were obtained previously from *P. nigrum* and *Piper retrofractum*, respectively [147,148]. These compounds together to piperine (**298**) showed toxic effects against third instar larvae of *Aedes* exposed for 48h, giving LC_{50} of 0.92, 0.89, 0.10, 0.0391, and 5.10ppm, respectively, to **279**, **280**, **281**, **282**, and **298**, respectively [146]. In addition, pipernonaline (**283**), isolated from *Piper longum* fruit, also showed toxicity against the fourth larval stage with LC_{50} value of 0.25ppm [149].

Subsequently, a number of compounds chemically related were found in a variety of extracts from *P. nigrum* that exhibited insecticidal activity against fourth instar of *Aedes* mosquito. Pipgulzarine (**284**) and pipzorine (**285**) had LC_{100} value of 6.0 and 70.0ppm, respectively [150]. Pipnoohine (**286**), pipyahyine (**287**), [(2*E*,4*E*)-octadienoyl]-*N*-isobutlyamide (**288**), sarmentine (**289**), [(2*E*,4*E*)-dodecadienoyl]-**N**-isobutylamide (**290**), [(2*E*,4*E*)-dodecadienoyl]-pyrrolidine (**291**), pellitorine (**292**), hexadecanoylpyrrolidine (**293**), [(2*E*)-octadecanoyl]-pyrrolidine (**294**), 1-[(2*E*,4*E*,12*Z*)-octadecanotrienoyl]-*N*-isobutylamide (**295**), 1-[7-(3,4-methylenedioxyphenyl)-(2*E*,4*E*)-heptadienoyl]-*N*-isobutylamide (**296**) presented toxic effects at 35, 30, 23, 27, 26, 29, 20, 75, 64, 29, and 13ppm, respectively [151]. The amide having highly extended conjugation piptigrine (**297**) had its structure elucidated by extensive NMR studies including 1D- and 2D-NMR and it exhibited toxicity of 15ppm against fourth instar larval stage [152]. Moreover, piperine (**298**), pipertipine (**299**), and pipercitine (**300**) had also showed toxicity against larvae, being **298** toxic at concentration of 10ppm [153].



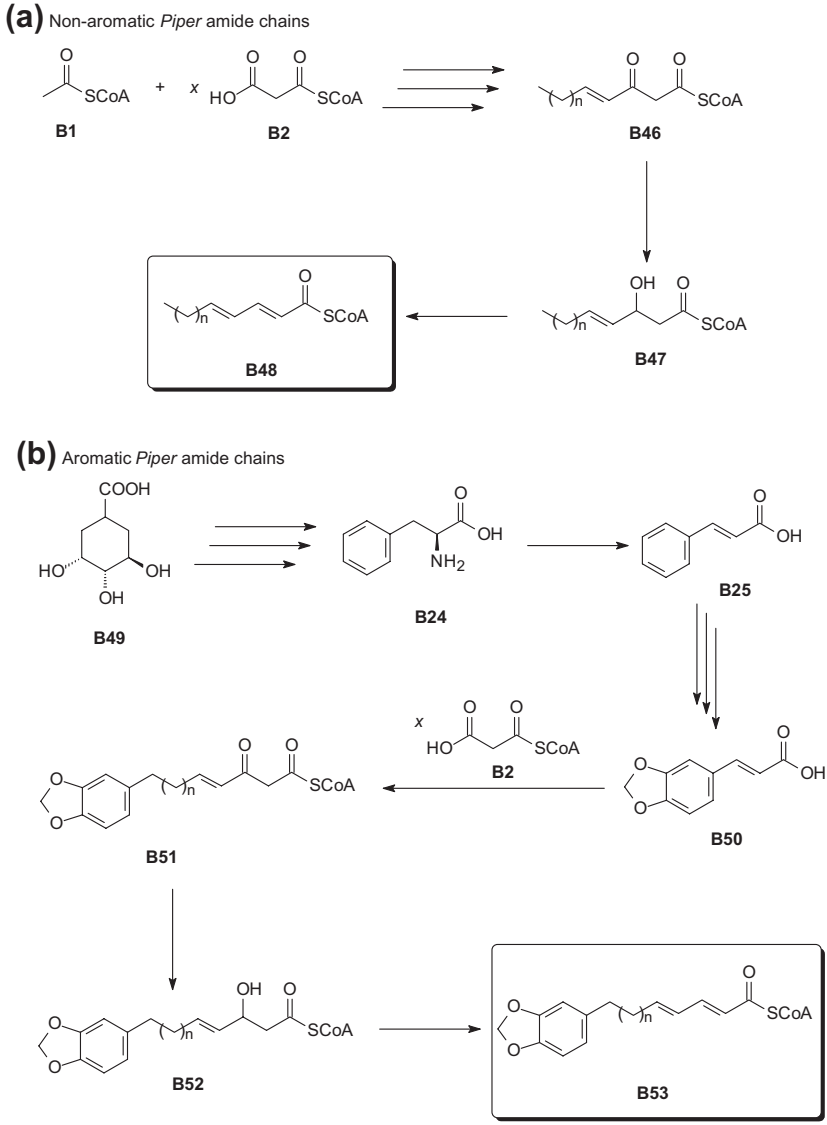


The continuous efforts to obtain potent insecticides led to studies on methanolic extract of dried ground seeds of *P. nigrum* resulted in the isolation and identification of two dimer-type diamides pipsaeedine (**301**) and pipbinine (**302**) that showed toxicity against fourth instar at 45 and 40ppm, respectively [154]. These compounds were obtained and identified together to the known insecticidal amides *tert*-butyldodecadienamide (**303**) (25ppm), *tert*-butylhexadecadienamide (**304**) (27ppm), piperanine (**305**) (17ppm), methylenedioxyphenylnonatrienoylpyrrolidine (**306**) (20ppm), and methylenedioxyundecatrienoylpiperidine (**307**) (25ppm) [154]. In addition Sidiqui and collaborators have been isolated the following amides pipwaqarine (**308**), [(2*E*,4*E*)-octadienoyl]-*N*-isobutylamide (**309**), [(2*E*)-hexadecanoyl]-pyrrolidine (**310**), [(2*E*,4*E*)-octadecadienoyl]-*N*-isobutylamide (**311**), (2*E*,4*E*)-eicosadienoyl-*N*-isobutylamide (**312**), retrofractamide A (**282**), and retrofractamide D (**313**) that exhibited toxicity at 30, 23, 64, 25, 25, 15, and 25ppm, respectively [155].



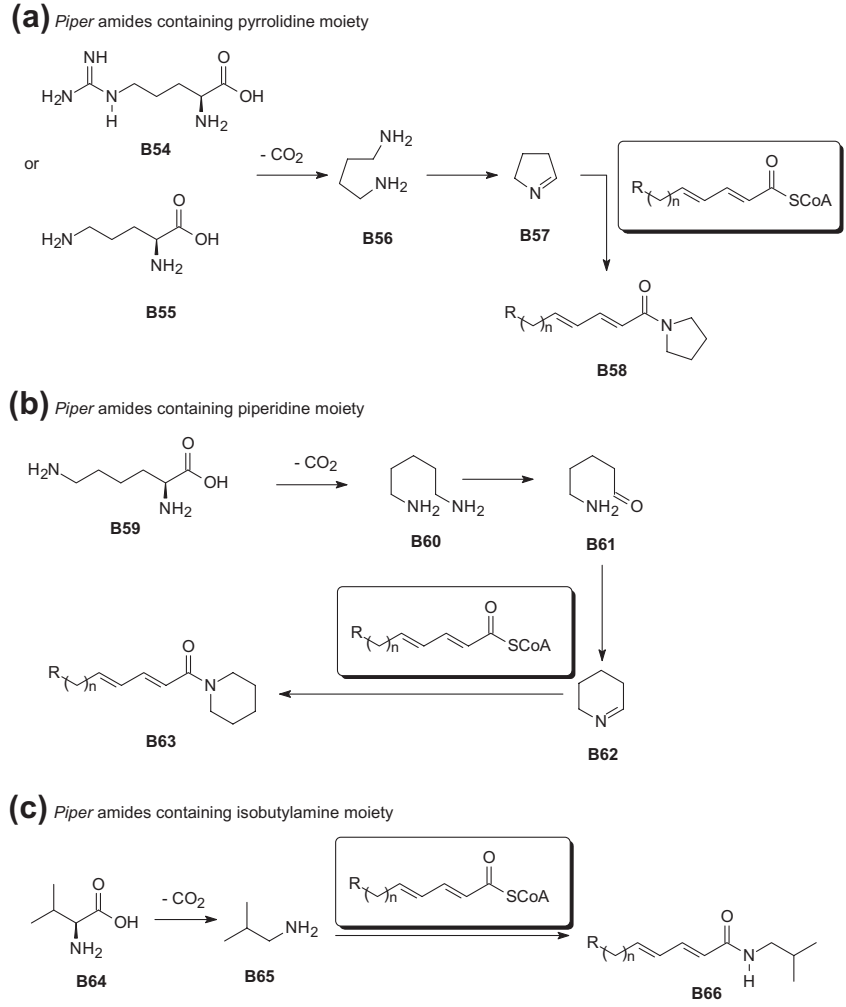
Piper amides are long-chain unsaturated amides constituted of an acyl derivative component and an amine moiety where isobutylamine, pyrrolidine and piperidine are predominant [156]. The long-chain unsaturated acyl component can be classified into two main structural types: (a) (2*E*,4*E*)-dienamides that are derived from straight-chain fatty acids (**B48**), probably biosynthesized through extension of the non-aromatic acyl precursor such as acetyl-thioester (**B1**) by malonyl-CoA (**B2**) [156,157]; and (b) piperonal unit (piperic acid CoA ester derivatives, **B53**) that comes from the phenylpropanoid shikimate-derived acyl precursor (**B50**) having chain extension by fatty acid-type, as shown in Scheme 7 [10,156,157].

Amine components are originated from aminoacids. Pyrrolidine ring (**B58**) can be biosynthesized from *L*-arginine (**B54**) or *L*-ornithine (**B55**) *via* putrescine (**B56**) by the loss of the carboxyl group and subsequent oxidative deamination into the aldehyde could produce the imine Δ^1-pyrroline (**B57**). Afterwards, nucleophyllic substitution reaction occurs in order to get the amide (Scheme 8).



SCHEME 7 Proposed biosynthetic pathway for the straight chain of *Piper* amides [10,156,157].

Piperidine moiety comes from *L*-lysine (**B59**) that produces cadaverin (**B60**) by descarboxylation; an oxidative deamination of α-amino nitrogen to obtain aldehyde (**B61**) and Schiff base formation (**B62**) are the next steps from this pathway to give this type of amide (**B63**). Finally, isobutylamine component (**B66**) would be derived from *L*-valine (**B64**) [10,156,157].



SCHEME 8 Proposed biosynthetic pathway for the amine components of *Piper* amides [10,156,157].

Studies on the structure–activity relationships between these amides and insects indicated that there are three essential points in the structures that increase the larvicidal effect: the conjugated dienamide chromophore (2*E*, 4*E* dienamide), chain length and isobutlyamine as the optimum amine moiety [146,158].

Miscellaneous

Repellency activity against mosquito *Ae. aegypti* was detected in a ethanolic solution containing dialdehyde rotundial (**314**), isolated from fresh leaves of

medicinal plant *Vitex rotundifolia* (Verbenaceae), showing to be efficient until 3h after the application (70% repellency) [158]. Comparative analyses with the known DEET indicated **314** as superior to the available commercial product [158]. In addition, amagnalactone (**315**), isolated together with **314** from fresh leaves of *Alberta magna* (Rubiaceae), showed repellent action, but shorter than DEET [159].

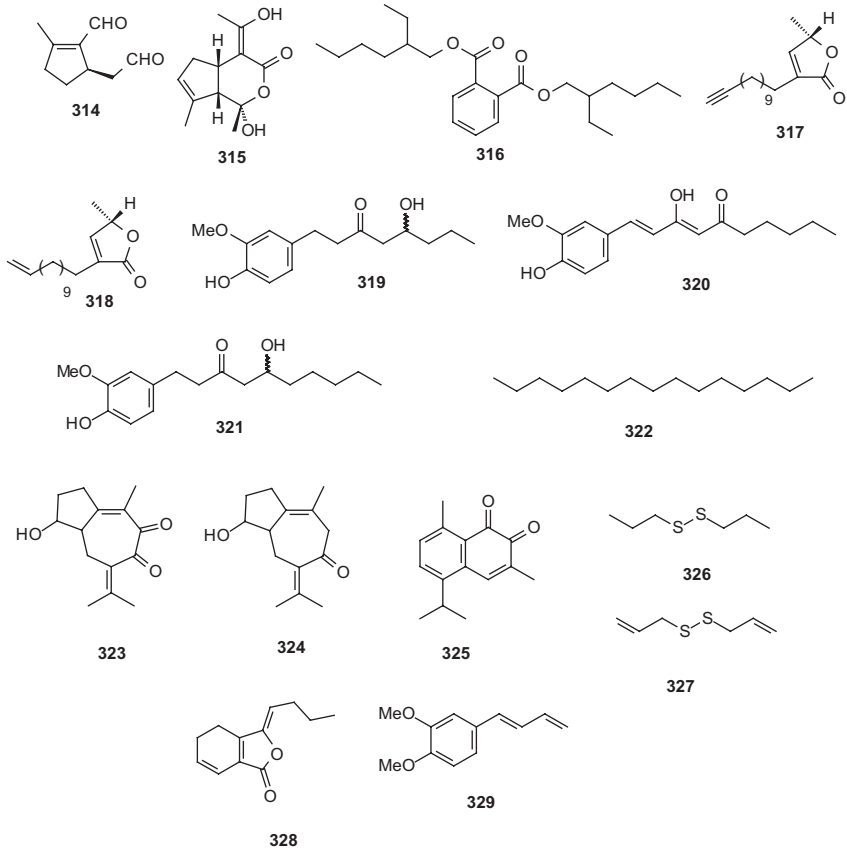
Fractionation of extract from *Sterculia guttota* (Sterculiaceae) seeds has led to the isolation of the bis-(2-ethylhexyl)-benezene-1,2-dicarboxylate (**316**) with activity against fourth instar larvae showing an LC_{50} of 79ppm [160]. Bioassay-guided fractionation of the dichloromethane extracts of the leaves from *Hortonia angustifolia*, *H. floribunda*, and *H. ovalifolia* (Monimiaceae) yielded the mosquito larvicidal butenolides (**317**) (LC_{50}=0.41ppm) and (**318**) (LC_{50}=0.47ppm) against second instar larvae of *Ae. aegypti* [161]. Moreover, Rahuman *et al.* have studied the larvicidal activity of petroleum ether extract of *Zingiber officinale* Roscoe (Zimgiberaceae), against early fourth instar *Aedes*. The LC_{50} values exhibited for 4-gingerol (**319**), 6-dehydrogingerdione (**320**) and 6-dihydrogingerdione (**321**) were 4.25, 9.80, 18.20ppm, respectively [162].

The alkane pentadecane (**322**), that showed larvicidal activity against third instar larvae of *Ae. aegypti* (LC_{50}=96.71ppm), was obtained from the methanolic extract of *A. heterotropoides* roots [75]. From the rhizomes of *Curcuma aromatica* (Zingiberaceae) two compounds, named 9-oxoneoprocurcumenol (**323**) and neoprocurcumenol (**324**), were isolated and both were active against third instar larvae with LC_{50}/LC_{90} of 5.81/9.99 and 13.63/23.75ppm, respectively [163].

Mansonone C (**325**) was isolated from the heartwood of *M. gagei* (Sterculiaceae) and assayed against the larvae of mosquito exhibiting an LD_{100} of 6.25ppm [67]. Major volatile organosulfur compounds from seeds of *A. indica* (Meliaceae) di-*n*-propyl disulfide (**326**) and diallyl disulfide (**327**) were tested against third instar larvae of *Ae. aegypti* and their toxicity were represented by means of LD_{50} equal to 6 and 66ppm, respectively [164].

Wedge *et al.* have reported the bioassay-guided fractionation of the chloroform extract from the roots of *Angelica sinensis* (Apiaceae) that resulted in the isolation and identification of the mosquito feeding-deterrent compound (*Z*)-ligustilide (**328**) [87]. Mosquito feeding-deterrent activity bioassay was performed by application of compounds **328**, callicarpenal (**223**), isolated previously from *Callicarpa* species [121], and DEET to cloth at a concentration of 25nmol/m^2 cloth. The proportion of mosquitoes not biting for (*Z*)-ligustilide (**328**) and callicarpenal (**223**) were 0.79 and 0.91, respectively, while DEET had 0.67 of mosquitoes not biting [87].

Bioassay-directed chromatographic fractionation of the dichloromethane extract of *Zingiber purpureum* Roscoe (Zingiberaceae) using second instar of *Aedes* (LC_{50} 4.76ppm) resulted in the isolation of 4-(3',4'-dimethoxyphenyl) buta-1,3-diene (**329**) that was tested against the bruchid *Callosobruchus maculates* showing ovicidal activity [165]. However, the pure compound was not tested against *Aedes* mosquito.



NATURAL INSECTICIDES DERIVED FROM FUNGI

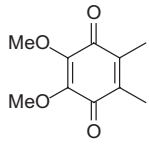
Polyketides

A metabolite of the fungus *Sesquicillium candelabrum* F-114 was isolated and identified as 2,3-dimethoxy 5,6-dimethyl-benzoquinone (**330**), also named aurantioglioclad in, obtained previously from *Gliocadium roseum* cultures, [166]. This compound showed an activity against second instar larvae of *Ae. aegypti* with LC_{50} of 23.0ppm [167].

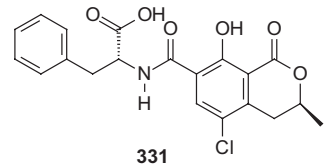
The known pentaketide ochratoxin A (**331**), isolated previously from *Aspergillus ochraceus* [168] and afterwards from several species of the fungal genera *Aspergillus* and *Penicillium* [169], was evaluated for larvicidal activity and exhibited an LD_{90} value of 10ppm [170]. However, its use as larvicidal is impracticable due to its nephrotoxic, immunosuppressive, teratogenic, and carcinogenic properties, leading to serious health hazard to animals and humans [169].

The non-aketide aurovertin B (**332**) was isolated from cultures of the fungal strain *Calcarisporium arbuscula* F-80 and had its LC_{50} registered larvicidal effect against second instar larvae was 8.7ppm [167].

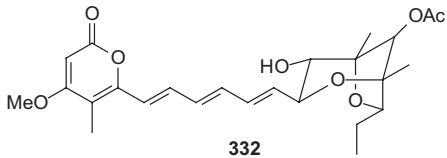
Xanthonol (**333**), a dimeric xanthone, was isolated during the screening for novel antiparasitic agents from a non-sporulating fungus MF6460 and had its structure elucidated by spectral analysis. This bis-xanthone exhibited larvicidal activity against first instar of *Ae. aegypti* with LD_{90} value of 8µg/mL [171].



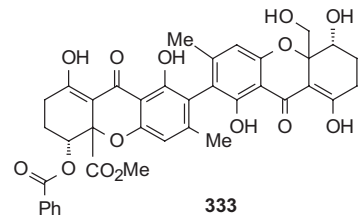
330



331



332

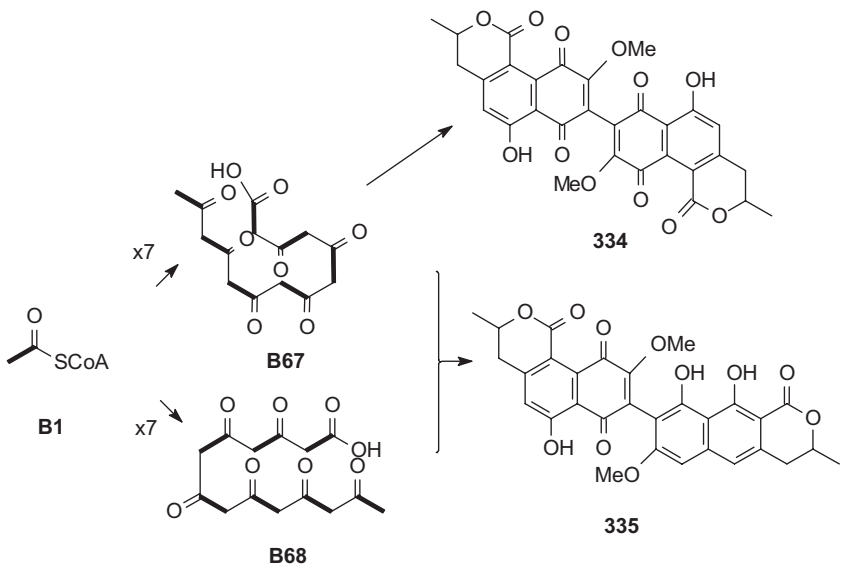


333

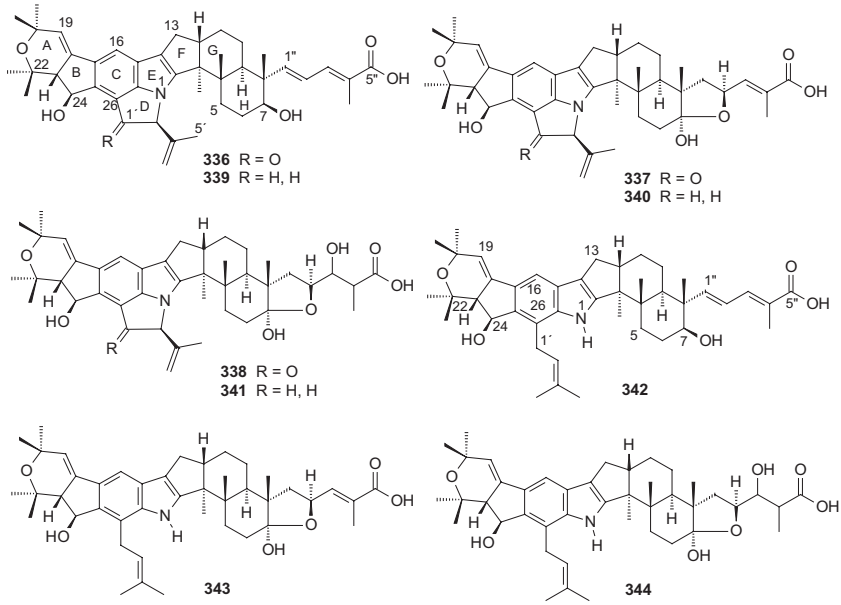
The fungal pigments xanthomegnin (**334**) and viomellein (**335**) have been isolated from several species of fungi [172]. They are dimeric naphtoquinones whose biosynthetic studies and NMR data confirmed their polyketide origin as shown in Scheme 9 [173]. Viomellein (**335**) is derived from 14 intact acetate units (**B1**) with formation of two intermediates **B67** and **B68**, arising from alternate folding of a common heptaketide chain, followed by introduction of C_1 units O-methylation and oxidative coupling. Xanthomegnin (**334**) is derived from coupling of two naphtoquinone moieties [173]. These compounds were evaluated for insecticidal activities against fourth instar larvae and exhibited an LC_{90} of 10ppm each [170].

Terpenoids

Nodulisporic acid (**336**) isolated from fermented culture of endophytic fungus *Nodulisporium* sp. was active against *Ae. aegypti* showing an LC_{50} of 0.5ppm [174]. Its structure was determined mainly by NMR methods, including Dunkel's computerized 2D INADEQUATE analysis. In addition, the relative stereochemistry of **336** was elucidated by ROESY, NOESY, NOEDS, transformation products, and further confirmed by X-ray analysis [174]. Subsequently, a number of compounds chemically related to **336** were found in cultures of this fungus. They are nodulisporic acid A1 (**337**), A2 (**338**) [175], B (**339**), B1 (**340**), B2 (**341**), [176]), C (**342**), C1 (**343**), and C2 (**344**) [177]. Compounds **339**–**341** belong to the 1'-deoxynodulisporic acids series [176], and **342**–**344** possess an opened D-ring [177].



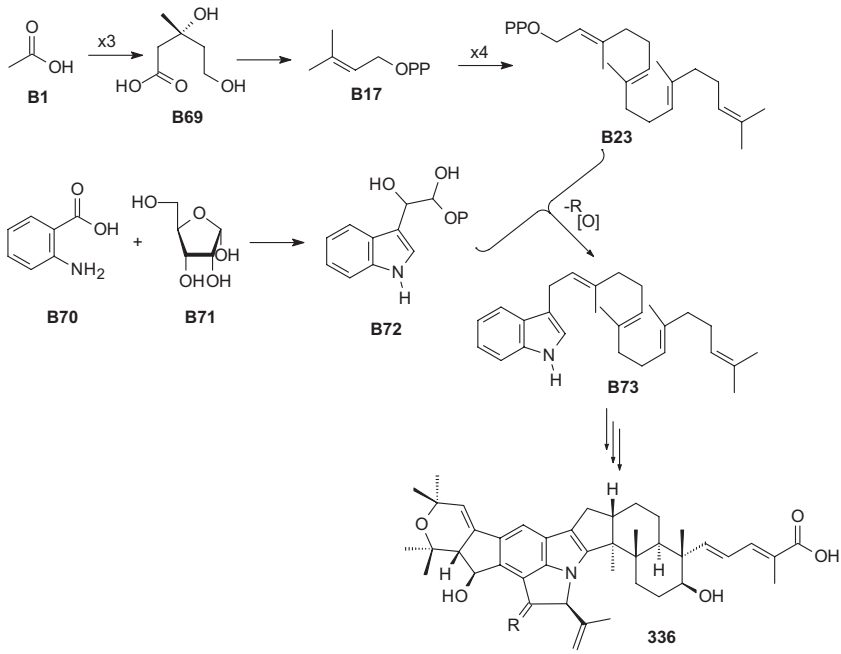
SCHEME 9 Proposed biosynthetic origin for the naphtoquinones **334** and **335.** Adapted from [173].



Nodulisporic acid (**336**) and its congeners belong to the new class of indole diterpenes, which can be characterized by several chemical features: **336** lacks the tertiary hydroxyl group at C-9 that is implicated in the tremorgenic properties of the related alkaloids, and contains a unique cyclopenthyl ring and a

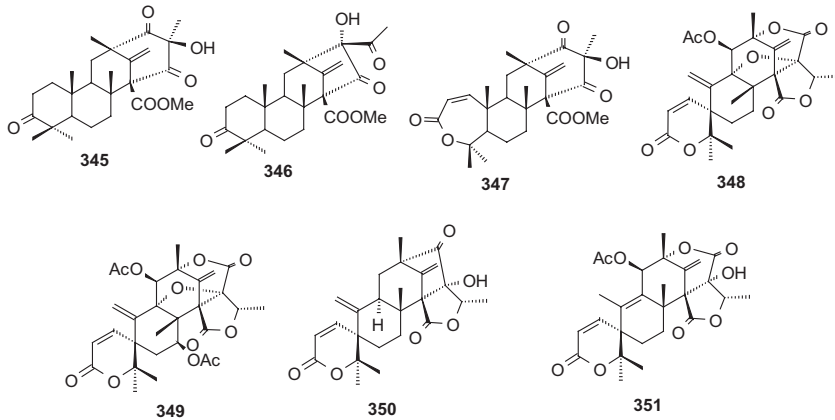
highly constrained β-keto-dihydropyrrole ring moieties [178]. Larvicidal activity assay against fourth instar were performed testing the compounds **336–338**, and **342**, which exhibited, respectively, the following LD_{90} 0.5, 0.2, 0.8 and 10ppm. **337** showed the highest activity against *Ae. aegypti* larvae, while **342** was significantly less active in this class of compounds [177]. A possible mode of action would be similar to the mechanism that **336** exerts its biological activity against *Drosophila melanogaster* by selectively opening of a glutamate-gated chloride channel, thus paralyzing it without affecting the mammalian host [179]. Moreover, a possible relationship between structure of **336** and its high larvicidal activity could be attributed to a substitution of the hydroxyl group at C-7 by a five-membered tetrahydrofuran ring, the non-polar the hemiketal moiety, while the hydration of the side chain double bound led to reduction of the activity at **338**.

Biosynthetic precursors involved in the biosynthesis of **336** were established by labelling **336** with ^{13}C-acetate and ^{13}C-mevalonate [178]. It demonstrated the classical acetate/mevalonate pathway: the intermediate **B73** arises from condensation of four isoprene units (**B17**) to produce the diterpene moiety (**B23**) followed by condensation with an indole nucleus that arises from anthranilic acid (**B70**) having incorporated ribose (**B71**) to produce indole-3-glycerol phosphate (**B72**) (Scheme 10). Subsequent three additional isoprenylations were incorporated to give **336** on the carbons C-10, C-17, and C-25 [178].



SCHEME 10 Proposed biosynthetic origin for the nodulisporic acid **336**. Adapted from [178].

Chemical studies on the constituents of *Penicillium* sp., when cultivated over sterilized rice, led to the isolation and structural elucidation of a series of meroterpenoids: preaustinoid A (**345**), preaustinoid B (**346**), preaustinoid A2 (**347**), dehydroaustin (**348**), acetoxydehydroaustin (**349**), and neoaustin (**350**) [180–182]. Based on the chemical-structure comparison, these compounds could be precursors of the meroterpenoid austin (**351**), the general biosynthesis route including a forel *Baeyer–Villiger*-like oxidation and other structural rearrangements [183]. Third instar larvae of *Ae. aegypti* were exposed to these meroterpenoids at a concentration of 500ppm each. Compounds **348** and **349** exhibited *in vitro* larvicidal activities of 100 and 70%, respectively, after 24h of exposure. Compound **351** displayed a very low larval mortality, and the other congeners being inactive. Statistical analysis was used to determine the lethal concentrations (LC_{50} values) of the bioactive compounds: dehydroaustin (**348**) and acetoxydehydroaustin (**349**) exhibited LC_{50} values of 2.9 and 7.3ppm, respectively. Notably, compound **348** caused larval mortality almost instantaneously, i.e., within less than 10min [184]. The larvicidal activity displayed by the meroterpenoids **348** and **349** is probably related to the δ-spirolactone system. Moreover, the additional acetoxy group in **349** seems to significantly reduce the larvicidal activity. Further, the very low activity of **351** compared to **348** and **349** suggests that the additional bridging furan ring in the latter two compounds also significantly influences activity. This could indicate a hydrophobic binding/reactivity site in this part of the molecule. Finally, compounds **345**–**347** with 'intact' (non-spiro) *A*-rings or an *A*-ring ε-lactone (as in **347**) did not show any larvicidal effect, further supporting the significance of a spiro structure [184].



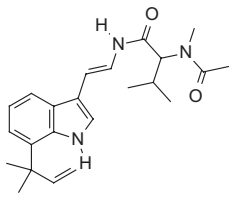
Others

Monitoring the fractionation guided by *in vitro* assay against fourth larval instars from the methylethylketone extract of *Aspergillus melleus* afforded isolation of mellamide (**352**), a indole amide, in addition to the known compounds

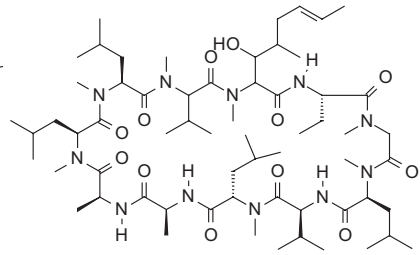
ochratoxin A (**334**), viomellin (**335**), and xanthomegnin (**331**) [170]. The dimethyl allyl substitute indole and the *N*-methyl, *N*-acetyl valine unit in **352** were assigned by spectroscopic methods. It exhibited a moderate activity of LD_{90} at 50ppm [170].

Treatment of larvae with methanolic extract from *Tolypocladium tundrense* and *T. terricola* induced a high level of mortality accompanied by vacuolization and subsequent destruction of mitochondria of the midgut cells. Cyclosporin A (**353**), a cyclic peptide, was identified as a predominant metabolite forming the spore surface layer [185]. In addition, the polypeptide tolypin, obtained from *Tolypocladium cylindrosporium*, *Tolypocladium inflatum*, and *Tolypocladium niveum* [186,187], caused a 100% of mortality against fourth instar of *Ae. aegypti* at a concentration of 1ppm within 24h. This metabolite is characterized as a mixture of homologous α-amino isobutyric acid peptides [185].

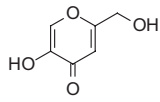
The known metabolite derived directly from glucose, kojic acid (**354**) [188] isolated from culture of *Aspergillus funiculosus* showed a larvicidal action exhibiting LC_{50} values for third and fourth instar larvae of 204.51 and 271.64ppm, respectively [189].



352



353



354

Fungal Extracts

Although out of the manuscript's scope, it is necessary to emphasize the need to carry on searching for new insecticidal metabolites from bioactive fungal extracts. For example, screening for biological active compounds from natural sources produced 125 extracts obtained from 57 different species belonging to 21 families of European fungi, from which only four dichloromethane extracts from *Albatrellus confluens*, *Hygrophorus poetarum*, *Agaricus xanthoderma*, and *Xerocomus badius*, showed activity against second instar larvae of *Ae. aegypti* [190]. However, no one subsequent study was found in the literature reporting isolated larvicidal compounds from those fungi. Moreover, investigation of larvicidal activity in the extract from *Penicillium citrinum* led to isolation of a compound having a molecular mass of 626Da that showed an LC_{50} of 15.8ppm against second instar larvae [191], but its structure has not been elucidated until now.

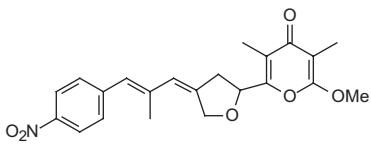
Matsumura and Knight reported the toxicity of aflatoxins mixture against *Ae. aegytpi*, against both larval and adult stage: they observed slight reduction in egg production and viability for the former while the mosquito exhibited reduced fecundity and fertility [192]. However, this class of compounds is carcinogenic and then would have limited practical use as insecticides.

NATURAL INSECTICIDES DERIVED FROM BACTERIA

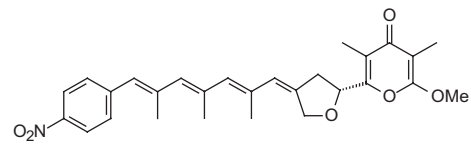
Polyketides

Some nitroaryl-substitute polyketides were found in cultures of several strains of *Streptomyces* spp. Aureothin (**355**) was first produced by *Streptomyces thioluteus* and had its structure completely elucidated on the basis of reactions and derivatives preparations [193,194]. Other species from this genus are also producers of **355**, including *Streptomyces spectabilis* and *Streptomyces griseus* var. *autotrophicus* [195]. Further isolation studies led to the discovery of spectinabilin (**356**), also named neoaureothin from cultures of *S. spectabilis* and *S. griseus* var. *autotrophicus* [195,196], and griseulin (**357**) from *S. spectabilis*, *S. griseus* var. *autotrophicus*, *Streptomyces luteoreticuli*, and *Streptoverticillium mobaraense* [195,197]. The structure of **357** was revised by Ishibashi *et al.* through spectroscopic analysis, and its data were identical to luteoreticulin [198].

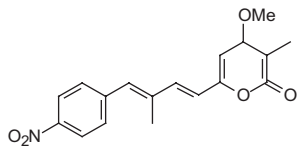
Beyond their biological activities such as nematocidal, antitumoral, and antimicrobial [195,199], these compounds also had larvicidal action against fourth instar of *Aedes* mosquito larvae causing 100% mortality at a concentration of 6.25ppm each [195].



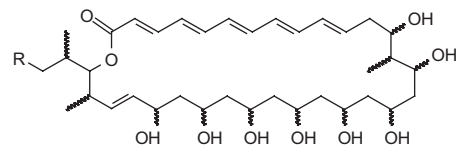
355



356

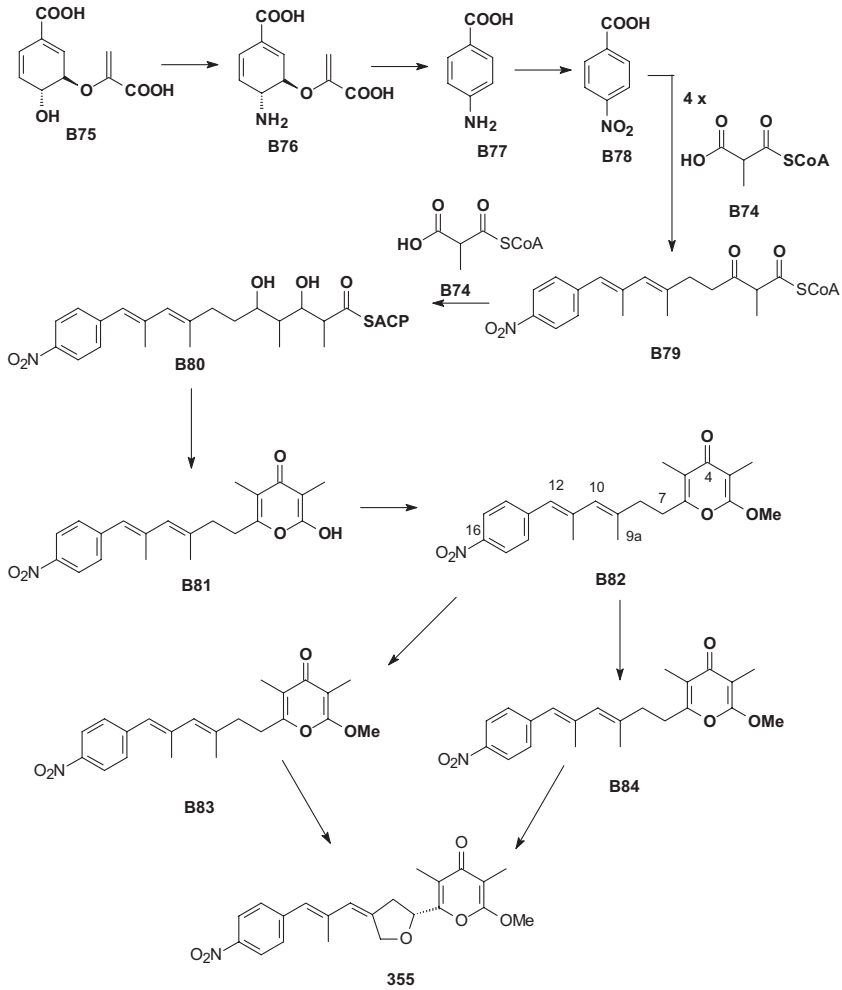


357



358 R = H
359 R = Me

Biosynthetic studies confirmed a mixed polyketide biosynthetic pathway for **355** which has the *p*-nitrobenzoate (PNBA, **78**) as PK starter unit and five methylmalonyl-CoA extenders (**B74**) [199,200]. **B78** is derived from *N*-oxidation mediated by *N*-oxygenases-like of *p*-aminobenzoate (PABA, **B77**), which in turn is synthesized from chorismate (**B75**) *via* 4-amino-4-deoxychorismate (**B76**) [199] as shown in Scheme 11. After the polyketide backbone is formed



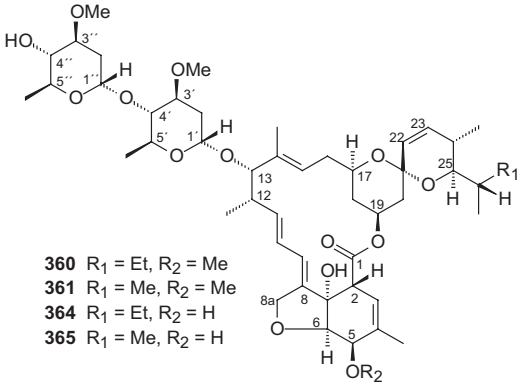
SCHEME 11 Proposed pathway for the biosynthesis of aureothin (**355**). Adapted from [199] and [200].

(including the pyrone-ring formation, **B81**) two subsequent events should be followed in order to give **355**: the pyrone methylation to produce the intermediate deoxy-aureothin (**B82**), followed by oxidation of two carbons – C-9a (**B83**) and C-7 (**B84**) – to form the homochiral five-membered heterocycle by a multifunctional cytochrome P450 monooxigenase (AurH) [200]. In addition, biosynthetic pathway for spectinabilin (**356**) is closely related to **355**, differing only in the number of diene moieties [201,202].

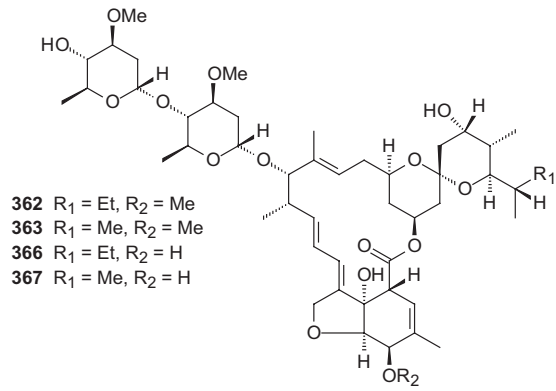
Faeriefungin is an inseparable mixture 1:1 of two compounds faeriefungin A (**358**) and faeriefungin B (**359**) [203]. These polyol polyene macrolide lactones were produced by *S. griseus* var. *autotrophicus*, and showed very potent antimicrobial activities (including fungi, gram-positive and gram-negative bacteria), as well as, a mosquitocidal activity against first instar of *Ae. aegypti* having LC_{50} of 100ppm. Structures of **358** and **359** were elucidated by spectroscopy techniques, including TOCSY experiments; the apparent polyketide biosynthetic route was proposed based on labelled acetate and propionate precursors added to the fungal culture [203].

Avermectin is a complex obtained from cultures of *Streptomyces avermitilis* MA-4680 during screening for anthelmintic activity was highly active against a variety of nematodes [204]. Their eight components, designated as avermectin A1a (**360**), A1b (**361**), A2a (**362**), A2b (**363**), B1a (**364**), B1b (**365**), B2a (**366**), and B2b (**367**), were isolated from intensively chromatographic procedures [205], being **360**, **362**, **364**, and **366** the major components. Their structures were established by mass spectrometry and ^{13}C NMR spectroscopy data as oleandrose disaccharide derivatives of pentacyclic 16-membered lactones [206] and confirmation of their structures as well as the relative and absolute stereochemistries establishment were obtained from X-ray crystallography [207]. Moreover, these compounds were named in according to the following denotation: *A*-series have a methoxyl group at the carbon C-5, whereas the *B*-series have a hydroxyl group; the 1-components have a double bond between carbons 22 and 23, whereas the 2-components have a single bond and a hydroxyl group at C-23; the *a*-components have a secondary butyl side chain at the carbon C-25, whereas the *b*-components have an isopropyl group [208,209]. Besides, if the literature not stated *a*- and *b*-series for the avermectins, it means that they occur as a mixture in a ratio approximately of 80:20 of *a*- and *b*-series, respectively [208,209].

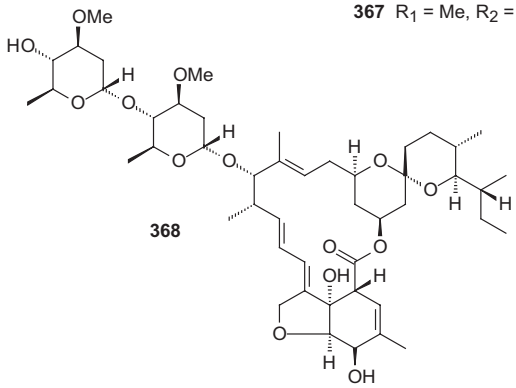
Due to B1 homologues possess the highest activity against a broad array of nematodes, medicinal chemistries synthesized ivermectin – 22,23-dihydroavermectin B1 (**368**) by selective hydrogenation of the 22–23 double bond of avermectin B1a (**364**) [209].



360 R_1 = Et, R_2 = Me
361 R_1 = Me, R_2 = Me
364 R_1 = Et, R_2 = H
365 R_1 = Me, R_2 = H



362 R_1 = Et, R_2 = Me
363 R_1 = Me, R_2 = Me
366 R_1 = Et, R_2 = H
367 R_1 = Me, R_2 = H



368

Two preparation commercially available containing avermectins were assayed against *Aedes* mosquito larvae (at the late third and early fourth instars) and both were effective at very low dosages [210]. Ivomec (MK-933, constituted of **368**) killed 50% of larvae at 23.41ppb (LC_{90} of 105.37ppb), while Abamectin (MK-936), consisting of a mixture 80:20 of avermectins B1a (**B19**) and B1b (**B20**), exhibited higher activity with LC_{50} and LC_{90} of 10.40 and 47.86ppb, respectively [210].

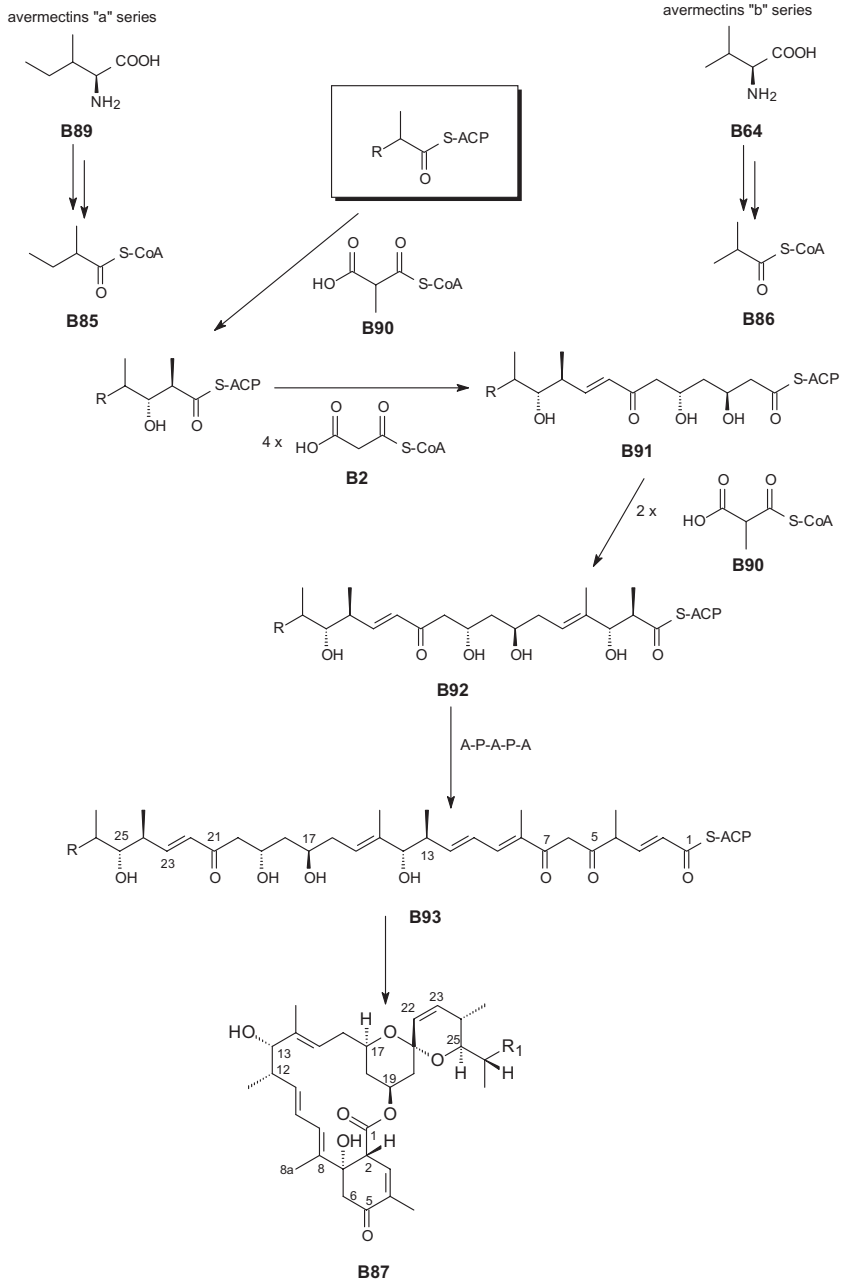
Biosynthetic pathways of avermectins have been extensively investigated by several researchers' groups. Two excellent reviews, written by Ikeda and

Omura and by Yoon *et al.*, show the main steps of the proposed biosynthetic route, which was deduced by labelling studies, identification and conversion of intermediates, and *in vitro* studies of enzymes [211,212]. Briefly, the avermectin biosynthesis can be classified into four stages: (1) biosynthesis of starter units (**B85** and **B86**); (2) formation of the polyketide backbone (6,8a-seco-6,8a-deoxy-5-oxoavermectin aglycons, **B87**) by polyketide synthases (PKSs); (3) pos-polyketide modification to obtain avermectin aglycons, and (4) glycosylation of avermectin aglycones using deoxythymidine diphosphate (dTDP)-L-oleandrose (**B88**) to give avermectins [211,212].

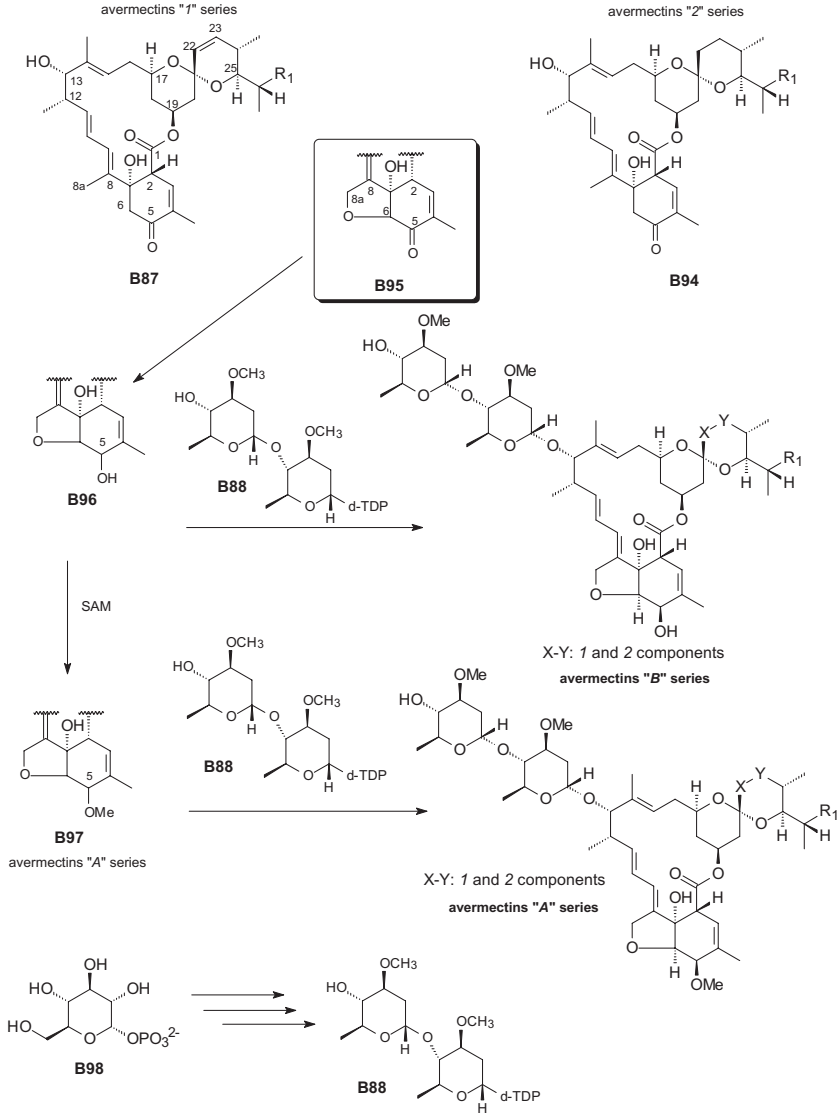
The starter units are derived from 2-methylbutyrate or isobutyrate, that arise from catabolism of *L*-isoleucine (**B89**) or *L*-valine (**B64**) aminoacids, to yield, respectively, the "*a*" and "*b*" series of avermectins as shown in Scheme 12 [212]. Biosynthesis of avermectin aglycons occurred by the addition of seven acetate units (A) from malonyl-CoA (**B2**) and five propionate units (P) from methylmalonyl-CoA (**B90**) to the one of starters acyl group in the following order: P–A–A–A–A–P–P–A–P–A–P–A to give the intermediate 6,8a-seco-6,8a-deoxy-5-oxoavermectin aglycons (**B87**). The first post-polyketide modification is suggested to be dehydration at C-22–C-23 (**B94**), differing ever since the avermectins *1* and *2* series, followed by the furan ring formation at carbons C-6–C-8 (**B95**), and then reduction of the keto group at carbon C-5 to give avermectin *B* aglycons (**B96**). Subsequent methylation by *O*-methyltransferases produces avermectin *A* series (**B97**), as seen in Scheme 13 [211–213]. *O*-methylation of *L*-oleandrose units (**B88**) by SAM should occur before incorporation of the sugar moiety into the aglycon.

Screening for new antiinsectan compounds of fermentation broth from the soil actinomycete *Saccharopolyspora spinosa* led to the isolation of a series of macrolides, designed as A83543 complex, but now named spinosyns A (**369**), B (**370**), C (**371**), and D (**372**) [214,215]. Their structures were elucidated by several techniques including mass spectrometry, 1-D and 2-D NMR data, and X-ray as a tetracyclic lactones consisting of a 12-membered macrocyclic lactone fused to a 5,6,5-*cis-anti-trans* tricyclic ring system to which are attached an aminosugar (*D*-forosamine) on the C-17 hydroxyl group and a neutral sugar (*L*-2', 3',4'-tri-*O*-methylrhamnose) on the C-9 hydroxyl group [214]. Spinosyn A (**369**) exhibited a highly potent activity against diverse larvae mosquitoes [215]. Since then, a number of additional macrolides analogues have been further isolated from cultures of this bacterium (from wild-types and mutants) [214,215], as well as the butenyl-spinosyns from *Saccharopolyspora pogona* [216,217]. The differences between these metabolites are in methyl substitution patterns on the forosamine nitrogen, at C6, C16, and C21 positions of the tetracyclic ring, and on 2,3',4'-tri-O-methylrhamnose [214,215,218,219]. In addition, the most abundant component in the fermentation is spinosyn **369** and the second is spinosyn **372**, which is 6-methyl-spinosyn A [218,219].

Despite a number of spinosyns analogues have exhibited some larvicidal activity, the most active compound is **369**, and followed by **372** [214,218]



SCHEME 12 Proposed pathway for the biosynthesis of avermectins: part 1: formation of the intermediate **B87**. Adapted from [211] and [212].

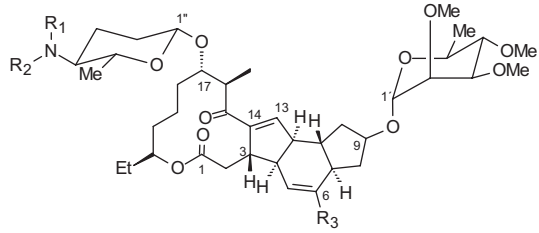


SCHEME 13 Proposed pathway for the biosynthesis of avermectins: part 2: formation of the avermectins backbone. Adapted from [211–213].

A natural mixture of both (~85:15%), obtaining for optimization of fermentation procedures, constitute the active principle of several commercial products by the name of spinosad (Tracer®, Success®, SpinTor®), the first product line produced by Dow Agrosciences that have been used for insect control due to its potency and broad-spectrum activity, including Lepidoptera, Diptera, and some Coleoptera [219–221]. Spinosad acts by causing rapid excitation

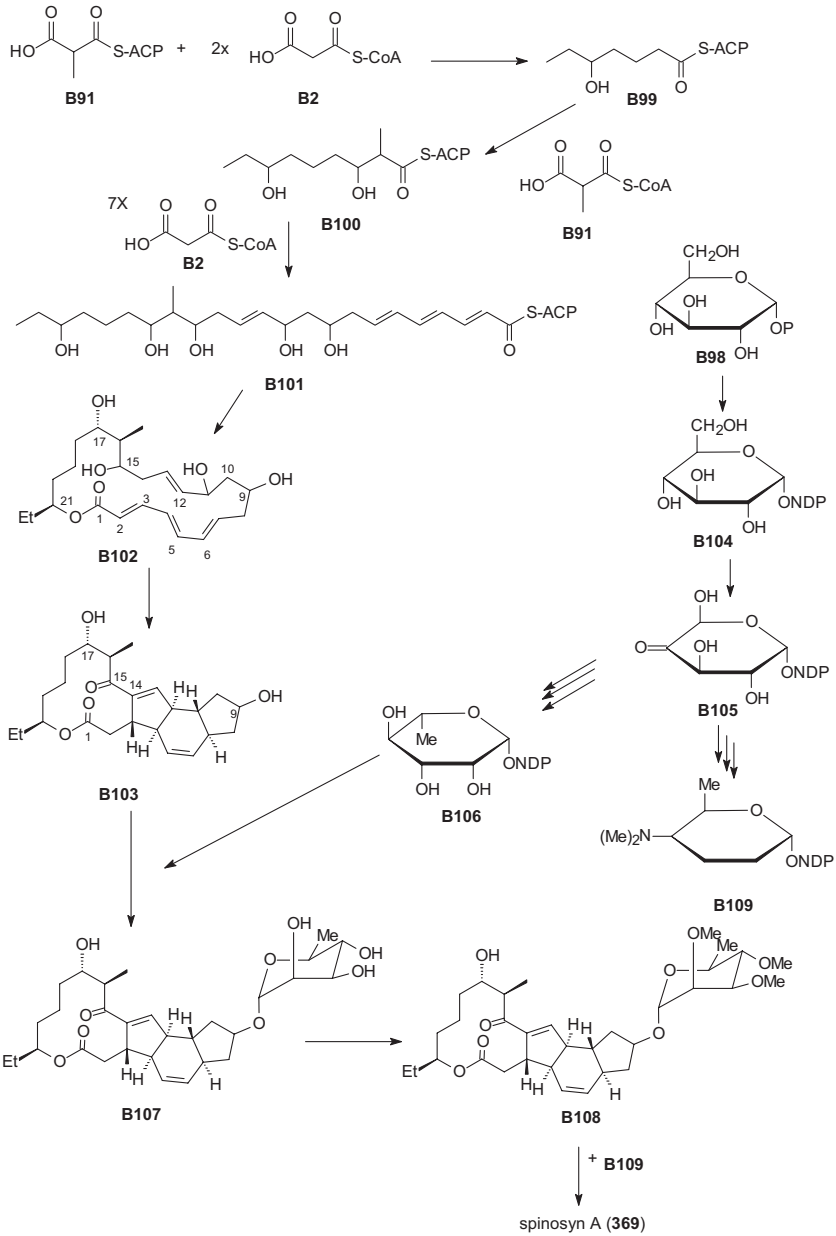
of the insect nervous system, probably through the interaction and binding at the nicotinacetylcholine (NAch) and δ-amino butyric acid receptors, leading to involuntary muscle contractions, prostration with tremors, and paralysis [219,220].

A suspension of spinosad (Tracer®) was evaluated against third and fourth instar of *Ae. aegypti* larvae exhibiting a 24h lethal concentration (LC_{50}) at 0.025ppm, a highly toxic formulation that could be used for treatment of mosquito breeding sites [222,223]. Moreover, field trials demonstrated that spinosad at a concentration of 10ppm inhibited the reproduction of mosquito for the entire 22-week period, while temephos provided complete inhibition for a period of eight weeks [222]. Applications of 1 and 5ppm spinosad or temephos in the water-filled containers used for flowers in a cemetery in Mexico resulted in complete control of mosquito development for 6, 8 and 10 weeks, respectively, being spinosad as effective as temephos granules in eliminating larval stages of *Aedes* [223]. By using wild-caught population of this mosquito in the assay, the value of LC_{50} was 0.060ppm, which is higher than the previous value that used the laboratory-reared Rockefeller strain [224].



369 $R_1 = R_2 = Me$, $R_3 = H$
370 $R_1 = H$, $R_2 = Me$, $R_3 = H$
371 $R_1 = R_2 = R_3 = H$
372 $R_1 = R_2 = R_3 = Me$

Biosynthetic pathway of spinosyns was based on the feeding studies, labelled compounds incorporation and determination of the spinosyn biosynthetic gen cluster revealing their polyketide origin, as shown in Scheme 14 [218,225,226]. Type 1 PKSs enzymes and a probable thioesterase cleavage of the polyketide chain are responsible by formation of the 22-membered monocyclic macrolide (**B102**) from nine acetate (A) (**B2**) and two propionate (P) (**B91**) units, in the following order: P–A–A–P–A–A–A–A–A–A–A. Subsequent intracellular cyclizations by three intramolecular carbon–carbon bonds gave aglycone (**B103**). Afterwards, the dTDP-rhamnose (**B106**) moiety is added to this aglycone (**B103**), forming a pseudoaglycone (**B108**) after by subsequent rhamnose methylation, and finally, glycosylation of pseudoaglycone by forosamine (**B109**) moiety produced spinosyn A (**369**). Both rhamnose and forosamine are synthesized from NDP-4-keto-6-deoxy-*D*-glucose (**B98**) and the methyl groups are derived from SAM [218,225,227,228].

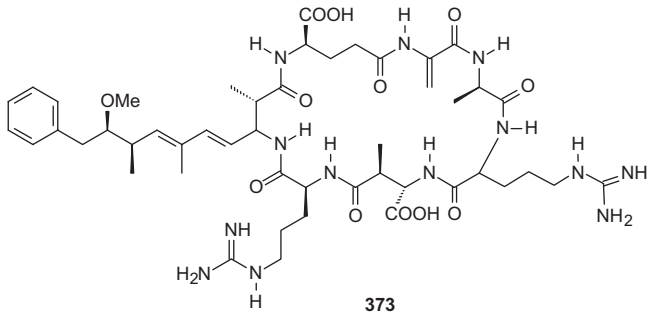


SCHEME 14 Proposed pathway for the biosynthesis of spinosyn A (**369**). Adapted from [218] and [225,226].

Spinosyn D (**372**) is likely formed by incorporation of propionate instead of acetate at the module 8 during the polyketide assembly to give the polyketide backbone ([221].

Peptides

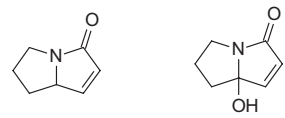
Previous study based on the search for novel mosquito larvae active compounds, revealed the extracts from the cyanobacterium *Microcystis aeruginosa* as the most effective against third or fourth instar larvae of *Aedes* [229]. Subsequent fractionation led to the isolation of [Dha^7] mycrocystin-RR (**373**), whose structure was assigned by aminoacid analysis, mass spectrometry and NMR spectroscopy [229]. Lethal concentration that killed 50% larvae was determined and the values after 24 and 48h of exposure were 22 and 15ppm, respectively [229]. This metabolite belongs to the cyclic peptide hepatotoxins class termed microcystins that have the general structure cyclo-(-*D*-Ala-*L*-*X*-erythro-β-methyl-*D*-*iso*Asp-*L*-*Y*-Adda-*D*-*iso*Glu10-*N*-methyldehydroAla), where Adda refers to the β-aminoacid residue of 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid and *X* and *Y* represent variable *L*-aminoacids; the term Dha refers to dehydroalanine [230].



373

Alkaloids

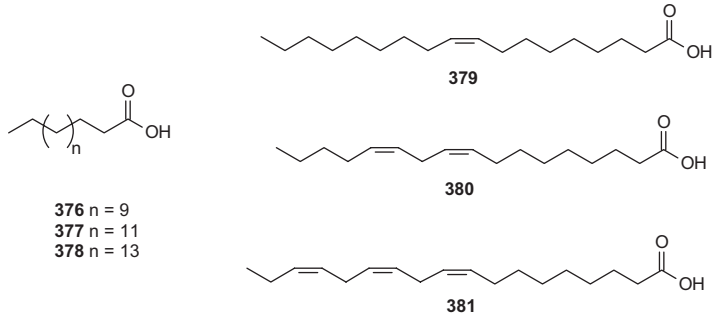
Two pyrrolizine derivatives 5,6,7,7a-tetrahydro-3H-pyrrolizin-3-one (**374**) and 5,6,7,7a-tetrahydro-3H-pyrrolizin-7a-ol-3-one (**375**) were isolated from shaken cultures of *Streptomyces globisporus* 0234 and *S. griseus* LKS-1 and the larvicidal activity of these compounds was evaluated in a mixture (1:2 ratio) against fourth instar larvae (in solutions there are interconvertions) with an LC_{50} of 112ppm [231].



374 **375**

Others

A mixture of unsaturated fatty acids, including oleic (**376**), linoleic (**377**), and γ-linolenic (**378**) acids, and saturated acids such as myristic (**379**), palmitic (**380**), and stearic (**381**) acids constitute the toxic fraction against second instar larvae of *Ae. albopictus*, a mosquito taxonomically close to *Ae. aegypti* [232]. These compounds were yielded by cyanobacterium *Oscillatoria agardhii* and they were identified by comparison to standards through GC–CI–MS analysis and those unsaturated might be the responsible for the larvicidal activity [232].



376 n = 9
377 n = 11
378 n = 13

379

380

381

The cyanobacterium *O. agardhii* was highly toxic to second and fourth instar of *Aedes* with values of LC_{50} of 4.0 and 8.7ppm, respectively, but the toxin was partially purified and its structure has not been elucidated until now [233]. Methanolic extract of the bio-fertilizer cyanobacterium *Westiellopsis* sp. was evaluated as regards larvicidal activity against fourth instar larvae, from which an LC_{50} of 55.84 and 3.95mg/L were detected after 24 and 48h exposure, respectively [234]. Finally, *Pseudomonas fluorescens*, a typical Gram-negative Eubacteria member, was isolated from dead second larvae *Ae. aegypti* and then cultivated on laboratory to give a bioactive fraction that killed these larval after 20min of exposure. Further studies are required to identify this exotoxin [235].

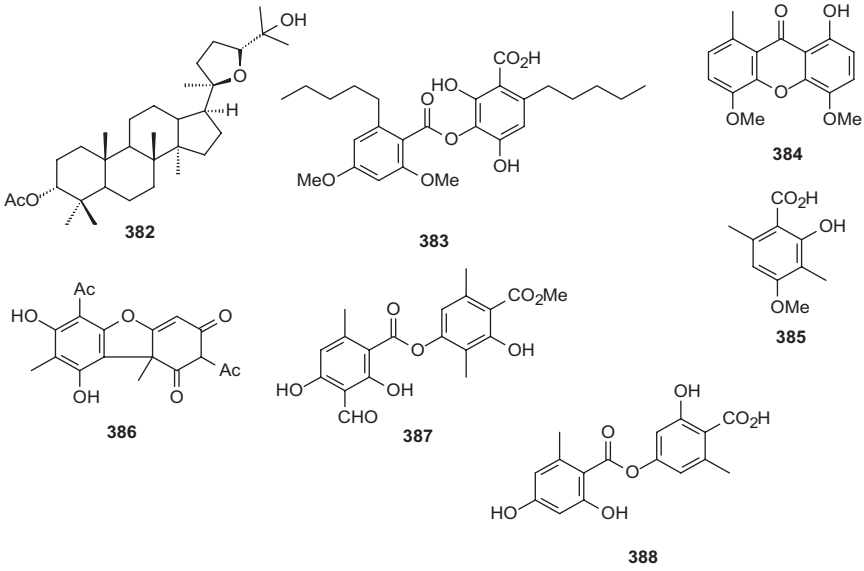
NATURAL INSECTICIDES DERIVED FROM OTHER ORGANISMS

Lichens are symbiotic associations between fungi and algal or cyanobacterial. Search for biologically active compounds of tropical lichens led to the isolation of cabraleadiol monoacetate (**382**), 4-*O*-methylcryptochlorophaeric acid (**383**), lichexanthone (**384**) from hexane extract of *Pyxine consocians*, and 3,6-dimethyl-2-hydroxy-4-methoxybenzoic acid (**385**) from *Heterodermia leucomelos* [236]. Compounds **381**, **384** and **385** led approximately 90% of moribund effect against second instar larvae after 24h of exposition, while **383** killed 60% of them [236]. Usnic acid (**386**) is a unique lichen metabolite found in several genera such as *Alectoria*, *Cladonia*, *Usnea*, *Lecanora*, *Ramalina*,

and *Evernia* [237]. Besides of inumerous biological activities attributed to **386**, this compound also had larvicidal activity against *Ae. aegypti* with LC_{50} and LC_{90} of 6.61 and 8.36ppm, respectively. However, due to its toxic effect against *Artemia salina*, **386** is not safe to use as larvicide, unless synthetic derivatives without toxicity can be designed [237].

Preliminary analysis of the larvicidal activity against second larval stage from extracts of macrolichen *Parmotrema pseudotinctorum* revealed 100% of mortality at a concentration of 20ppm and 50% of mortality at 5ppm in some extracts [238]. Comparative thin layer chromatography indicated the presence of atranorin (**387**) and olecanoric acid (**388**) in these extracts, but the attribution of the insecticidal activity to these compounds should be made only after their isolation and larvicidal bioassay [238], since **387** isolated previously had no effect against *Aedes* larvae [236].

Sponges can also be a source to obtain leads of insecticidal molecules against *Aedes* mosquitoes. The sponge extracts of *Psammaplysilla purpurea* and *Haliclona cribricutis* showed LC_{50} values at 25.9 and 31.46ppm, respectively, against fourth instar larvae [239].



BIOCONTROL AGENTS

Biological control of *Ae. aegypti* can also be made by entomopathogens microorganisms (bacteria and fungi), as well as predators and parasites. Thus, various biological control agents have been investigated with the support of United Nations Development Programme, World Health Organization Special Program for Research and Training in Tropical Diseases (WHO/TDR) [240].

Microorganisms

Bacteria used for mosquito control are spore-forming bacteria (*Bacillus*) such as *Bacillus sphaericus* and *B. thuringiensis* var *israelensis* (Bti) H-14 [8]. Both bacilli produce crystalline proteinaceous toxins during sporulation which is activated after ingested by mosquito larvae leading to changes in their gut [8]. *Bacillus sphaericus* (strain 2362) is the active ingredient in Vectolex® being the most widely used commercial mosquitocidal to all known *Culex* species, and to many important anopheline species such as *Anopheles gambiae* (the key malaria vector in Africa). It also had effect to many *Aedes* species, excepting *Ae. aegypti* that showed resistance [2,241,242].

Nowadays, temephos (**1**) and Bti are the most used larvicides in control programs against dengue's vector [8]. This bacteria was discovered by Ishiwata in 1901 during his study of a bacterial disease of silkworms and its insecticidal activity was described in the literature by Berliner in 1951 [243]. In addition, continuing studies to find new strains with novel insecticidal spectra led to the discovery of two new important subspecies of *B. thuringiensis*: *B. thuringiensis* var. *israelensis* (H-14, also known as Bti) and *B. thuringiensis* var. *morrisoni*. The former has excellent larvicidal activity against several mosquitoes [243], and has being used against *Ae. aegypti* since 1980 [8]. Several commercial formulations are now available in the market for use against mosquito vectors. Laboratory evaluation of Teknar HP-D [244], Vectobac® and Bacticide® [241] against *Ae. aegypti* showed great susceptibility of the mosquito to this three formulations. Cessation of feeding activity, accompanied by paralysis, was observed in *Ae. aegypti* larvae 30–45min after exposure to Bti-Teknar HP-D [244]. Moreover, a 100% reduction in the density of third and fourth instars of *Ae. aegypti* was observed when exposed them to Vectobac® and Bacticide® [241]. These bacterial agents are highly effective against mosquito larvae at very low doses and completely safe to other non-target organisms, environment, man and wild life, and are suitable for community use [240]. The high toxicity of the whole spore crystal complex is due to a synergistic interaction between the 25kDa protein and one or more other proteins which are produced during sporulation and transformed into toxins under specific conditions after ingestion by larvae [240,245]. However, a limiting factor of Bti lays on its short time of action, often no more than a few days [8].

Fungal entomopathogens (those that can infect insects) represent one of the most important way of controlling insect populations in nature, as well as practical and commercially useful means for biological control of insect pests (including insects vector of viral or bacterial pathogens) [246–249]. After tarsal contact, spores of these fungi adhere to and penetrate the cuticle, grow internally, producing a series of toxins that kill the mosquito [250]. Moreover, these toxins inside the host can inhibit the insect's immune system, modify the insect's behaviour, or act as post-mortem antibiotics against competing microorganisms [251]. Thus, these fungi are considered promising sources of novel biologically active compounds, including insecticidal, antitumoral, and antibiotics [249,251].

The largest numbers of fungal species that are pathogenic to insects belong to the order Hypocreales (Dikarya, Ascomycota, Pezizomycotina, Sordariomycetes, Hypocreomycetidae) [249]. About 13 entomopathogenic fungal species or subspecies have been developed as commercial biopesticides, although the currently available products are based on *Beauveria*, *Metarhizium*, *Lecanicillium* and more rarely, *Isaria* strains [249]. However, not any available mycoinsecticide showed effect against *Ae. aegypti*. There are some studies reported in the literature pointed to the four main genera of fungi having different actions against the mosquito: *Metarhizium*, *Isaria*, *Paecilomyces*, and *Lecanicillium*. They exhibited adulticidal, larvicidal, and ovicidal activity [252–254]. Besides, conidia of the fungus *B. bassiana* produced 100% mortality in adults of *Ae. aegypti* within 5 days after exposure, but had had no effect to the larvae [255]. However, larvae treated with blastospores of *B. bassiana* showed 85% mortality after 96h post-exposure [256].

Other Organisms

Larvae of *Ae. aegypti* have been successfully controlled with predatory copepods (a group of small crustaceans), which are feasible to achieve massive reductions in disease transmission through biological control of vector mosquitoes, killing mainly first instar of *Ae. aegypti* [257].

The control of the vector in water jars can be carried out using fish predators. *Poecilia reticulata*, *Tilapia mossambica*, and *Sarotherodon niloticus* and *Carassius auratus auratus* had a significant reduction in the number of larvae, while the larvivorous fish *Trichogaster trichopterus* led to complete reduction for a period of 9 months [8,258].

An aquatic hemipteran bug *Diplonychus indicus* [259] and flatworms (Turbellaria) [260] also showed to be efficient predators of larvae in many laboratory studies.

BIOASSAY AGAINST *AEDES AEGYPTI* LARVAE

Exploring the natural resources to find new leads which have insecticidal activity against the vector *Ae. aegypti* is an urgent task once dengue incidence keeps on increasing. Screening programmes using any insecticidal assay protocols would contribute to the discovery of different and useful new bioactive compounds. It can also be included in the known natural products since many of them have not been subjected to testing for control of the *Aedes* mosquito. Moreover, *Ae. aegypti* is commonly used in insecticide screening programmes because it is usually less susceptible and it is easy to colonize in the laboratory [261]

Tables 1–5 report those natural products produced by plants (Tables 1 and 2), fungi (Table 3), bacteria (Table 4) and lichen (Table 5) which have been evaluated for their insecticidal action. These tables show that different methodologies, including ovicidal, larvicidal and repellent activities, were used to verify

TABLE 2 Toxicity of Plant Compounds Against *Aedes aegypti*

Comp.	Values of			Effect against *Ae. aegypti*	Ref.
	LC_{50} ppm, 24h (48h)	LC_{90} ppm, 24h (48h)	LC_{100} (LD_{99}) ppm, 24h (48h)		
11	100[a], 8.8[b]	35.39[b]	–	[a,b]Larvicidal (early fourth instar); [c]repellent	[a][39], [b][40], [c][41]
12	100[a], 18.20[b]	96.33[b]	–	Larvicidal (early fourth instar)	[a][39], [b][40]
13	–	–	0.2	Larvicidal (second instar)	[42]
14	–	–	0.3	Larvicidal (second instar)	[42]
15	–	–	2.0	Larvicidal (second instar)	[42]
16	–	–	1.0	Larvicidal (second instar)	[42]
17	–	–	2.0	Larvicidal (second instar)	[42]
18	–	–	1.0	Larvicidal (second instar)	[42]
19	141	–	–	Larvicidal (third or early fourth instar)	[43]
20	30.4	–	–	Larvicidal (third or early fourth instar)	[43]
21	75.7	–	–	Larvicidal (third or early fourth instar)	[43]
22	8.29	–	–	Larvicidal (third or early fourth instar)	[43]
23	3.04	–	–	Larvicidal (third or early fourth instar)	[43]
24	9.5	22.3	–	Larvicidal (third instar)	[46]

Continued

TABLE 2 Toxicity of Plant Compounds Against *Aedes aegypti*—Cont'd

Comp.	Values of			Effect against *Ae. aegypti*	Ref.
	LC_{50} ppm, 24h (48h)	LC_{90} ppm, 24h (48h)	LC_{100} (LD_{99}) ppm, 24h (48h)		
25	–	–	0.5	Larvicidal (first, second instar)	[49]
26	0.019[b]	0.039[b]	0.5[a]	[a]Larvicidal (first, second, and fourth instar); [b]larvicidal (fourth instar)	[a][49], [b] [50]
27	–	–	0.5	Larvicidal (first instar)	[49]
28	–	–	0.5	Larvicidal (first instar)	[49]
29	–	–	0.5	Larvicidal (first instar)	[49]
30	0.079[b]	0.16[b]	0.5[a]	[a]Larvicidal (first, second, and fourth instar); [b]larvicidal (fourth instar)	[a][49], [b] [50]
31	–	–	67–99% mortality at 0.5ppm	Larvicidal (first instar)	[49]
32–34 (mixture)	3.9	4.56	–	Larvicidal (third instar); toxicity to adult aedes (male and female)	[52]
35	–	–	–	Larvicidal (third instar)	[56]
36	–	–	–	Larvicidal (third instar)	[57]
37	–	–	–	Larvicidal (third instar)	[57]
38	75–100	–	–	Larvicidal (third instar)	[58]

39	19.4	–	–	Larvicidal (third instar)	[59]
40	NA	–	–	Larvicidal (third instar)	[59]
41	NA	–	–	Larvicidal (third instar)	[59]
42	5.43	6.56	–	Larvicidal (fourth instar)	[60]
43	31.47	55.72	–	Larvicidal (fourth instar)	[60]
44	13.64	19.28	–	Larvicidal (fourth instar)	[60]
45	1.26	4.10	–	Larvicidal (fourth instar)	[60]
46	NA	NA	–	Larvicidal (fourth instar)	[60]
47	NA	NA	–	Larvicidal (fourth instar)	[60]
48	40.66	53.87	–	Larvicidal (fourth instar)	[60]
49	31.21	42.72	–	Larvicidal (fourth instar)	[60]
50	3.3	8.8	–	Larvicidal (fourth instar)	[62]
51	26.3	-	–	Larvicidal (third instar)	[64]
52	9.64	16.154	–	Larvicidal (fourth instar)	[64]
53	3.78	7.06	–	Larvicidal (fourth instar)	[64]
54	4.745	9.062	–	Larvicidal (fourth instar)	[64]
55	3.587	6.64	–	Larvicidal (fourth instar)	[64]
56	4.553	7.993	–	Larvicidal (fourth instar)	[64]

Continued

TABLE 2 Toxicity of Plant Compounds Against *Aedes aegypti*—Cont'd

Comp.	Values of			Effect against *Ae. aegypti*	Ref.
	LC_{50} ppm, 24h (48h)	LC_{90} ppm, 24h (48h)	LC_{100} (LD_{99}) ppm, 24h (48h)		
57	7.919	10.833	–	Larvicidal (fourth instar)	[64]
58	1.391	2.50	–	Larvicidal (fourth instar)	[64]
59	1.170	4.550	–	Larvicidal (fourth instar)	[64]
60	9.703	16.999	–	Larvicidal (fourth instar)	[64]
61	0.873	1.41	–	Larvicidal (fourth instar)	[64]
62	7.28	10.445	–	Larvicidal (fourth instar)	[64]
63	5.75	12.453	–	Larvicidal (fourth instar)	[64]
64	14.952	23.065	–	Larvicidal (fourth instar)	[64]
65	–	–	–	Larvicidal (second instar)	[65]
66	–	–	12.5	Larvicidal (second instar)	[65]
67	–	–	50	Larvicidal (second instar)	[65]
68	–	–	25	Larvicidal (second instar)	[65]
69	–	–	Not tested	Larvicidal (second instar)	[65]
70	–	–	12.5	Larvicidal (second instar)	[66]

71	–	–	25	Larvicidal (second instar)	[66]
72	–	–	12.5	Larvicidal (second instar)	[66]
73	–	–	6.25	Larvicidal (second instar)	[67]
74	–	–	–	Larvicidal (second instar) – 70% mortality at 100ppm	[68]
75	90	–	–	Larvicidal (third instar)	[71]
76	61	–	–	Larvicidal (third instar)	[71]
77	17	–	–	Larvicidal (third instar)	[71]
78	19	–	–	Larvicidal (third instar)	[71]
79	16	–	–	Larvicidal (third instar)	[71]
80	18	–	–	Larvicidal (third instar)	[71]
81	1.9	–	–	Larvicidal (early fourth instar)	[72]
82	19.6	–	–	Larvicidal (early fourth instar)	[72]
83	16.3	25.0	–	Larvicidal (early fourth instar)	[62]
84	15.4	23.7	–	Larvicidal (early fourth instar)	[62]
85	1.8	2.9	–	Larvicidal (third instar)	[73]
86	7.4	16.5	–	Larvicidal (third instar)	[73]
87	NA	NA	–	Larvicidal (third instar)	[73]

Continued

TABLE 2 Toxicity of Plant Compounds Against *Aedes aegypti*—Cont'd

Comp.	Values of			Effect against *Ae. aegypti*	Ref.
	LC_{50} ppm, 24h (48h)	LC_{90} ppm, 24h (48h)	LC_{100} (LD_{99}) ppm, 24h (48h)		
88	NA	NA	–	Larvicidal (third instar)	[73]
89	NA	NA	–	Larvicidal (third instar)	[73]
90	2.1	–	–	Larvicidal (third instar)	[74]
91	NA	–	–	Larvicidal (third instar)	[74]
92	10.49	–	–	Larvicidal (third instar)	[75]
93	–	–	17.6	Larvicidal	[76]
94	–	–	25	Larvicidal	[76]
95	–		NA	Larvicidal	[76]
96	2.37	–	–	Larvicidal (first instar)	[77]
97	10.8[a]	–	25[b]	[a]Larvicidal (third instar); [b]larvicidal	[a][78], [b][79]
98	11.5	–	–	Larvicidal (third instar)	[78]
99	11.0	–	–	Larvicidal (third instar)	[78]
100+101	–	–	100	Larvicidal	[79]
102	–	–	12.5	Larvicidal (second instar)	[80]

103	–	–	25	Larvicidal (second instar)	[80]
104	26.99	–	–	Larvicidal (third instar)	[75]
105	57.65	–	–	Larvicidal (third instar)	[75]
106	24.4	54.7	–	Larvicidal (third instar)	[81]
107	44.5[a], 88[b]	100[a]	–	Larvicidal (third instar)	[a][81], [b][82]
108	49	63.8	–	Larvicidal (third instar)	[81]
109	513	–	–	Larvicidal (third instar)	[82]
110	136	–	–	Larvicidal (third instar)	[82]
111	194	–	–	Larvicidal (third instar)	[82]
112	243	–	–	Larvicidal (third instar)	[82]
113	577	–	–	Larvicidal (third instar)	[82]
114	177	–	–	Larvicidal (third instar)	[82]
115	–	–	200	Larvicidal (fourth instar)	[90]
116	162.93[a], 55.47[b], 369.78[c]	–	–	[a]Ovicidal; [b]larvicidal (third instar); [c]against pupae	[91]
117	–	–	25	Larvicidal	[79]
118	–	–	NA	Larvicidal	[79]
119	0.082	0.152	–	Larvicidal (fourth instar)	[92]

Continued

TABLE 2 Toxicity of Plant Compounds Against *Aedes aegypti*—Cont'd

Comp.	Values of			Effect against *Ae. aegypti*	Ref.
	LC_{50} ppm, 24h (48h)	LC_{90} ppm, 24h (48h)	LC_{100} (LD_{99}) ppm, 24h (48h)		
120+121	45.77	–	–	Larvicidal (fourth instar)	[93]
120+122	62.23	–	–	Larvicidal (fourth instar)	[93]
123	–	–	45	Larvicidal (fourth instar)	[94]
124	2.23	–	–	Larvicidal (early fourth instar)	[95]
125	34.04	–	–	Larvicidal (early fourth instar)	[95]
126	77.99	–	–	Larvicidal (early fourth instar)	[95]
127	89.60	–	–	Larvicidal (early fourth instar)	[95]
128	>200	–	–	Larvicidal (early fourth instar)	[96]
129	>200	–	–	Larvicidal (early fourth instar)	[96]
130	8.23	13.23	–	Larvicidal (early fourth instar)	[96]
131	41.35	54.47	–	Larvicidal (early fourth instar)	[96]
132	>200	–	–	Larvicidal (early fourth instar)	[96]
133	191.45	–	–	Larvicidal (early fourth instar)	[96]
134	9.34	15.32	–	Larvicidal (early fourth instar)	[96]

135	54.13	74.28	–	Larvicidal (early fourth instar)	[96]
136	15.0	57.7	–	Larvicidal (third instar)	[46]
137	–	–	50	Larvicidal (third instar)	[98]
138	–	–	100	Larvicidal (third instar)	[98]
139	13.4	–	–	Larvicidal (third instar)	[99]
140	–	–	3	Larvicidal	[100]
141	–	–	50	Larvicidal	[100]
142	–	–	6	Larvicidal	[100]
143	–	–	6	Larvicidal	[100]
144	–	–	>50	Larvicidal	[100]
145	–	–	>50	Larvicidal	[100]
146	–	–	>50	Larvicidal	[100]
147	–	–	NT	Larvicidal	[100]
148	(16.15)	–	–	Larvicidal (second instar)	[101]
149	3.7	15.1	–	Larvicidal (late third instar)	[46]
150	0.122[a]	0.223[a]	–	Larvicidal (fourth instar)[a]; ovicidal activity	[92]
151	0.103[a]	0.191[a]	–	Larvicidal (fourth instar)[a]; ovicidal activity and 100% repellency	[92]

Continued

TABLE 2 Toxicity of Plant Compounds Against *Aedes aegypti*—Cont'd

Comp.	Values of			Effect against *Ae. aegypti*	Ref.
	LC_{50} ppm, 24h (48h)	LC_{90} ppm, 24h (48h)	LC_{100} (LD_{99}) ppm, 24h (48h)		
152	>20	–	–	Larvicidal (second instar)	[102]
153	>20	–	–	Larvicidal (second instar)	[102]
154	0.085[a]	0.171[a]	–	Larvicidal (fourth instar)[a]; 100% repellency against the mosquito and the strong oviposition deterrent effect with production of a 66.3% repellency at 2.0ppm	[92]
155	0.47	1.45	–	Larvicidal (late third instar);	[46]
156	(16.15)	–	–	Larvicidal (second instar)	[101]
157	3.1	36.6	–	Larvicidal (third instar)	[103]
158	(7.2)	–	–	Larvicidal (third instar)	[105]
159	10.8	–	–	Larvicidal (third instar)	[105]
160	7.6	–	–	Larvicidal (third instar)	[105]
161	10.7	–	–	Larvicidal (third instar)	[105]
162	NA	–	–	Larvicidal (third instar)	[105]
163	4.0	–	–	Larvicidal (third instar)	[105]

164	2.0	–	–	Larvicidal (third instar)	[105]
165	2.5	–	–	Larvicidal (third instar)	[105]
166	NA	–	–	Larvicidal (third instar)	[105]
167	0.47[a]	–	10[b]	Larvicidal ([a]second, [b]fourth instar)	[a][110], [b] [109]
168	>20	–	–	Larvicidal (second instar)	[110]
169	>20	–	–	Larvicidal (second instar)	[110]
170	1.6[a]	–	5[b]	Larvicidal ([a]second, [b]fourth instar)	[110]
171	1.4[a]	–	5[b] (95%)	Larvicidal ([a]second, [b]fourth instar)	[110]
172	NA	–	–	Larvicidal (second instar)	[110]
173	NT	–	–	Larvicidal (third instar)	[112]
174	>50	–	–	Larvicidal (third instar)	[112]
175	24.55	–	–	Larvicidal (third instar)	[112]
176	–	–	–	23% mosquito-repelling after 1h (1.5g/m^2)	[114]
177	–	–	–	92 and 75% Mosquito-repelling after 1 and 3h, respectively (1.5g/m^2)	[114]
178	–	–	–	94 and 82% Mosquito-repelling at 0.01 and 0.05mg/cm^2; 76% repellency at 0.4mg/cm^2 within 30min.	[41]
179	107	–	–	Larvicidal (third instar)	[78]

Continued

TABLE 2 Toxicity of Plant Compounds Against *Aedes aegypti*—Cont'd

Comp.	Values of			Effect against *Ae. aegypti*	Ref.
	LC_{50} ppm, 24h (48h)	LC_{90} ppm, 24h (48h)	LC_{100} (LD_{99}) ppm, 24h (48h)		
180	–	–	100	Larvicidal (third instar)	[115]
181	2.91	–	–	Larvicidal (fourth instar)	[116]
214	17	–	–	Larvicidal (third instar)	[81]
215	13	–	–	Larvicidal (third instar)	[81]
216	–	–	50	Larvicidal (fourth instar)	[119]
217	–	–	12.5	Larvicidal	[120]
218	–	–	50	Larvicidal	[120]
219	–	–	50	Larvicidal	[120]
220	–	–	NA	Larvicidal	[120]
221	–	–	NA	Larvicidal	[120]
222	–	–	–	Deterrent activity	[121]
223	–	–	–	Deterrent activity	[121]
224	–	–	–	Deterrent activity	[121]
225	–	–	–	Deterrent activity	[121]
226	–	–	–	100% Repellency at 78.6μg/cm^2	[122]

227	–	–	–	82.2% Repellency at 78.6μg/cm^2	[122]
228	36.2	–	–	Larvicidal (first instar);	[123]
229	10.0	–	–	Larvicidal (first instar);	[123]
230	88.30	–	–	Larvicidal (third instar)	[117]
231	–	–	(10)	Larvicidal (fourth instar)	[119]
232	0.8	8.2	–	Larvicidal (third instar)	[124]
233	87.3	128.8	–	Larvicidal (third instar)	[124]
234	NA	NA	–	Larvicidal (third instar)	[124]
235	NA	NA	–	Larvicidal (third instar)	[124]
236	50.08	87.31	–	Larvicidal (fourth instar)	[126]
237	14.69	27.50	–	Larvicidal (fourth instar)	[126]
238	21.76	25.13	–	Larvicidal (fourth instar)	[126]
239	186.21	–	–	Larvicidal (third instar)	[127]
240	82	–	–	Larvicidal (first instar);	[128]
241	4.4	–	–	Larvicidal (first instar);	[130]
242	18.6	–	–	Larvicidal (third instar)	[131]
243	25.1	–	–	Larvicidal (third instar)	[131]
244	27.9	–	–	Larvicidal (third instar)	[131]

Continued

TABLE 2 Toxicity of Plant Compounds Against *Aedes aegypti*—Cont'd

Comp.	Values of			Effect against *Ae. aegypti*	Ref.
	LC_{50} ppm, 24h (48h)	LC_{90} ppm, 24h (48h)	LC_{100} (LD_{99}) ppm, 24h (48h)		
245	104.7	–	–	Larvicidal (third instar)	[131]
246	NA	–	–	Larvicidal (third instar)	[131]
247	–	–	–	Larvicidal (63% mortality of third instar at 1000ppm)	[132]
248	0.625[a]	–	–	Larvicidal; mutagenic property[b]	[a][12],[b][133]
249	0.74[a]	–	–	Mutagenic property[b]	[a][12],[b][133]
250	53.0	–	–	Larvicidal (fourth instar)	[135]
251	2.14	–	–	Larvicidal (fourth instar)	[135]
252	21.0	–	–	Larvicidal (fourth instar)	[135]
253	83.0	–	–	Larvicidal (fourth instar)	[135]
254	–	–	100% at 50 and 10ppm	Larvicidal (fourth instar)	[136]
255	–	–	100% at 50ppm	Larvicidal (fourth instar)	[136]
256	–	–	–	Larvicidal (fourth instar, 50% mortality at 50ppm);	[136]
257	–	–	–	Larvicidal (fourth instar, 35% mortality at 50ppm);	[136]

258	–	–	–	Larvicidal (fourth instar, 30% mortality at 50ppm);	[136]
259	–	–	–	Larvicidal (fourth instar, 20% mortality at 50ppm);	[136]
260	–	–	–	Larvicidal (fourth instar, 40% mortality at 50ppm);	[136]
261	–	–	NA	Larvicidal (fourth instar)	[136]
262	–	–	NA	Larvicidal (fourth instar)	[136]
263	–	–	NA	Larvicidal (fourth instar)	[136]
264	13	–	–	Larvicidal (second instar)	[137]
265	71.6	–	–	Larvicidal (second instar)	[137]
266	217.1	–	–	Larvicidal (second instar)	[137]
267	14.51	–	–	Larvicidal (second instar)	[138]
268	27.47	–	–	Larvicidal (second instar)	[138]
269	4.052	–	–	Larvicidal (second instar)	[138]
270	69	–	–	Larvicidal (third instar)	[139]
271	4.8	–	–	Larvicidal (third instar)	[139]
272	–	–	20.9 (LC_{99})	Larvicidal (fourth instar)	[140]
273	–	–	100	Larvicidal (fourth instar)	[142]

Continued

TABLE 2 Toxicity of Plant Compounds Against *Aedes aegypti*—Cont'd

Comp.	Values of			Effect against *Ae. aegypti*	Ref.
	LC_{50} ppm, 24h (48h)	LC_{90} ppm, 24h (48h)	LC_{100} (LD_{99}) ppm, 24h (48h)		
274	–	–	12.5	Larvicidal (fourth instar)	[142]
275	–	–	12.5	Larvicidal (fourth instar)	[142]
276	3.6	–	–	Larvicidal (third instar)	[143]
277	2.1	–	–	Second instar	[144]
278	–	–	1000	Ovicidal and larvicidal	[145]
279	0.92		–	Larvicidal (third instar)	[146]
280	0.89	–	–	Larvicidal (third instar)	[146]
281	0.1	–	–	Larvicidal (third instar)	[146]
282	0.0391	–	–	Larvicidal (third instar)	[146]
283	0.25	–	–	Larvicidal (fourth instar)	[149]
284	–	–	6	Larvicidal (fourth instar)	[150]
285	–	–	70	Larvicidal (fourth instar)	[150]
286	–	–	35	Larvicidal (fourth instar)	[151]
287	–	–	30	Larvicidal (fourth instar)	[151]

288	–	–	23	Larvicidal (fourth instar)	[151]
289	–	–	27	Larvicidal (fourth instar)	[151]
290	–	–	26	Larvicidal (fourth instar)	[151]
291	–	–	29	Larvicidal (fourth instar)	[151]
292	–	–	20	Larvicidal (fourth instar)	[151]
293	–	–	75	Larvicidal (fourth instar)	[151]
294	–	–	64	Larvicidal (fourth instar)	[151]
295	–	–	29	Larvicidal (fourth instar)	[151]
296	–	–	13	Larvicidal (fourth instar)	[151]
297	–	–	15	Larvicidal (fourth instar)	[153]
298	5.1	–	10[b]	Larvicidal ([a]third [b]fourth instar)	[a][146], [153][b]
299	–	–	–	Larvicidal (fourth instar)	[153]
300	–	–	–	Larvicidal (fourth instar)	[153]
301	–	–	45	Larvicidal (fourth instar)	[154]
302	–	–	40	Larvicidal (fourth instar)	[154]
303	–	–	25	Larvicidal (fourth instar)	[154]
304	–	–	27	Larvicidal (fourth instar)	[154]
305	–	–	17	Larvicidal (fourth instar)	[154]

Continued

TABLE 2 Toxicity of Plant Compounds Against *Aedes aegypti*—Cont'd

Comp.	Values of			Effect against *Ae. aegypti*	Ref.
	LC_{50} ppm, 24h (48h)	LC_{90} ppm, 24h (48h)	LC_{100} (LD_{99}) ppm, 24h (48h)		
306	–	–	20	Larvicidal (fourth instar)	[154]
307	–	–	25	Larvicidal (fourth instar)	[154]
308	–	–	30	Larvicidal (fourth instar)	[155]
309	–	–	23	Larvicidal (fourth instar)	[155]
310	–	–	64	Larvicidal (fourth instar)	[155]
311	–	–	25	Larvicidal (fourth instar)	[155]
312	–	–	25	Larvicidal (fourth instar)	[155]
313	–	–	25	Larvicidal (fourth instar)	[155]
314	–	–	–	100% (1h), 70% (3h) Repellency mosquito	[158]
315	–	–	–	Repellency mosquito	[159]
316	79	–	–	Larvicidal (early fourth instar)	[160]
317	0.41	–	–	Larvicidal (second instar)	[161]
318	0.47	–	–	Larvicidal (second instar)	[161]
319	4.25	–	–	Larvicidal (early fourth instar)	[162]

320	9.80	–	–	Larvicidal (early fourth instar)	[162]
321	18.20	–	–	Larvicidal (early fourth instar)	[162]
322	96.71	–	–	Larvicidal (third instar)	[75]
323	5.81	9.99	–	Larvicidal (third instar)	[163]
324	13.63	23.75	–	Larvicidal (third instar)	[163]
325	–	–	6.25	Larvicidal	[67]
326	6	–	–	Larvicidal (third instar)	[164]
327	66	–	–	Larvicidal (third instar)	[164]
328	–	–	–	Mosquito feeding deterrent	[87]
329	4.76[a]	–	–	Larvicidal (second instar); [a]fraction containing **329**	[165]

TABLE 3 Toxicity of Fungi Metabolites Against *Aedes aegypti*

Comp.	Values of			Effect against *Ae. aegypti*	Ref.
	LC_{50}, ppm, 24h (48h)	LC_{90}, ppm, 24h (48h)	LC_{100} (LD_{99}), ppm, 24h (48h)		
330	23	–	–	Larvicidal (second instar)	[167]
331	–	10	–	Larvicidal (fourth instar)	[170]
332	8.7	–	–	Larvicidal (second instar)	[167]
333	–	8	–	Larvicidal (first instar)	[171]
334	–	10	–	Larvicidal (fourth instar)	[170]
335	–	10	–	Larvicidal (fourth instar)	[170]
336	0.5	0.5	–	Larvicidal (fourth instar)	[174,177]
337	–	0.2	–	Larvicidal (fourth instar)	[174,177]
338	–	0.8	–	Larvicidal (fourth instar)	[177]
339	–	NT	–	Larvicidal (fourth instar)	[177]
340	–	NT	–	Larvicidal (fourth instar)	[177]
341	–	NT	–	Larvicidal (fourth instar)	[177]

342	–	10	–	Larvicidal (fourth instar)	[177]
343	–	NT	–	Larvicidal (fourth instar)	[177]
344	–	NT	–	Larvicidal (fourth instar)	[177]
345	NA	–	–	Larvicidal (third instar)	[184]
346	NA	–	–	Larvicidal (third instar)	[184]
347	NA	–	–	Larvicidal (third instar)	[184]
348	2.9	–	–	Larvicidal (third instar)	[184]
349	7.3	–	–	Larvicidal (third instar)	[184]
350	NA	–	–	Larvicidal (third instar)	[184]
351	NA	–	–	Larvicidal (third instar)	[184]
352	–	50	–	Larvicidal (fourth instar)	[170]
353			97% Mortality	Larvicidal (fourth instar)	[185]
354	204.51[a]; 271.64[b]	–	–	Larvicidal (third instar[a] and fourth instar[b])	[189]

TABLE 4 Toxicity of Bacteria Metabolites Against *Aedes aegypti*

Comp.	Values of			Effect against *Ae. aegypti*	Ref.
	LC_{50}, ppm, 24h (48h)	LC_{90}, ppm, 24h (48h)	LC_{100} (LD_{99}), ppm, 24h (48h)		
355	–	–	6.25	Larvicidal (fourth instar)	[195]
356	–	–	6.25	Larvicidal (fourth instar)	[195]
357	–	–	6.25	Larvicidal (fourth instar)	[195]
358 + 359	100	–	–	Larvicidal (first instar)	[203]
364 + 365 (MK-936)	0.0104	0.0478	–	Larvicidal (third or fourth instar)	[210]
368 (MK-933)	0.0234	0.1053	–	Larvicidal (third or fourth instar)	[210]
369 + 372	0.025	–	–	Larvicidal (third or fourth instar)	[222,223]
373	22 (15)	–	–	Larvicidal (third or fourth instar)	[229]
374 + 375	112	–	–	Larvicidal (fourth instar)	[231]
376–378	–	–		Toxicity against second instar	[232]

TABLE 5 Toxicity of Metabolites Produced by Lichens Against *Aedes aegypti*

Comp.	Values of			Effect against *Ae. aegypti*	Ref.
	LC_{50}, ppm, 24h (48h)	LC_{90}, ppm, 24h (48h)	LC_{100} (LD_{99}), ppm, 24h (48h)		
382	–	–	–	90% Moribund effect against second instar larvae	[236]
383	–	–	–	60% Death against second instar larvae	[236]
384	–	–	–	90% Moribund effect against second instar larvae	[236]
385	–	–	–	90% Moribund effect against second instar larvae	[236]
386	6.61	8.36	–	Larvicidal activity	[237]
387	NA	–	–	–	[236]
388	NT	–	–	–	[238]

the potential of these compounds to combat the mosquito but the larvae toxicity assay was by far the most applied test (representing 93% of all insecticidal assay protocols with plant metabolites).

Larval mosquito susceptibility test consists of the exposition of mosquito larvae to solutions or emulsions of insecticides or potential candidates by immersion at a standard period of time, followed by counting the mortality [262]. Although any larval stage may be used in these tests (as observed in the tables), WHO recommends to perform the assay with older larvae (third and fourth stage) once they are more resistant to insecticides than young larvae (first and second stage); pupae do not feed and, thus, do not ingest the bioactive compounds [262]. However, Pridgeon *et al.* proposed a high-throughput screening method using first instar larvae (1-day-old after oviposition) in order to search for potential compounds that possess larvicidal activity by using a 24-well-plate having only 10μL of diluted chemicals in a final volume of 1mL [263]. The main advantages of this methodology are the possibility to screen large number of natural compounds and the facility in using freshly hatched *Ae. aegypti* larvae rather than more advanced larval stage which need to be reared and sorted.

The results are expressed by counting dead larvae (those remaining at the bottom or not capable of coming up to surface) and the mortality is then calculated as a percentage of dead larvae in such dosage of insecticidal in a set of experiments. The percentage of mortality is corrected by using statistical methods and the results are expressed as LC_{50} (lethal concentration 50 value is the concentration of a material that kill 50% of the tested larvae), LC_{90} (lethal concentration 90 value is the concentration of a material that kill 90% of the tested larvae), LC_{99} (lethal concentration 99 value is the concentration of a material that kill 99% of the tested larvae) or LC_{100} (lethal concentration 100 value is the concentration of a material that kill 100% of the tested larvae).

LC values of compounds expressed in Tables 1–5 were registered by using data obtained mainly after 24h of larvae exposition to them. LC registered after 48h are in brackets. The LC_{50} or LC_{90} values (expressed in ppm) were grouped as: highly active ($0 < LC < 10$), moderately active ($10 \leq LC < 50$), slightly active ($50 \leq LC \leq 100$), very slightly active ($LC > 100$) and inactive (no mortality was observed). It was observed some variability of LC_{50} values for the same compounds and it probably may be attributed to the different experimental conditions, such as susceptibility on the mosquito tested, differences in the number of replicates tested, and the operational assay conditions. For convenience the LC values of compounds found in these tables were expressed in ppm to provide a basis for comparison of their potencies, although for some chemicals these data were expressed in other units in their original papers and (converted) to ppm herein.

A possible way to overcome these differences lays on the establishment of a screening methodology that encompasses standardization on the number and larval stage used, number of replicates, time of exposure, solvents, etc. Special attention should be driven to the larval stage to be used and this choice would

be made based on the abilities on the research groups in sorting the larval stage recommend by WHO. If there is no cooperation between chemists and entomologists, a preliminary screening procedure using freshly hatched *Ae. aegypti* larvae as proposed by Pridgeon *et al*. [263] would be adopted and then for those compounds that exhibit any larvicidal effect should be tested with older *Aedes* larvae as well as other insects species.

There is the need to emphasize that the results obtained in laboratory conditions do not reflect directly the real consequence in the field. Thus, field trials should be performed for compounds that showed interesting larvicidal activity due to photo- and thermostability presented for some compounds. Moreover, the new potent larvicidal candidates will be accepted for application if they exhibit a very low toxicity to non-target organisms as well as being added to the drinking water reservoirs. From this, many candidates fell down due to their toxic properties considerably diminishing the number of available larvicidal agents to the next steps to introduce a new bioactive to the market. Therefore, an ideal larvicidal should be highly selective, biodegradable, thermo- and photo-resistant.

CONCLUSIONS

There are two methods of controlling or preventing dengue virus transmission: combating the virus and combating the vector. As long as there is no efficient vaccine for the prevention to dengue the only way to prevent it is through the vector control, and this combat should be guided by three axis: guiding and educating people for eliminating the breeding places, larvicidal application in water reservoirs and the use of insecticides in the endemic periods. "*Any reduction in mosquito vector reduces the average number of blood meals taken, and any reduction in the number of blood meals taken reduces the probability of the vector acquiring a disease agent, and subsequently transmitting it to other hosts*" [250].

Natural products research can contribute searching for useful compounds for dengue-vector control programmes. In this review the structures of compounds isolated from plants (319), fungi (25), bacteria (27) and lichen (07) have been reported, from which a total of 357 metabolites exhibited some activity against *Aedes* mosquito.

A total of 74 plant species have been shown to hold secondary metabolites which have significant insecticidal properties and they are distributed in 33 families from which the major occurrence are found in the families Rutaceae (9.5%), Leguminosae (8.1%), Annonaceae (6.7%), Piperaceae (6.7%), Zingiberaceae (6.7%), Asteraceae (5.4%), and Verbenaceae (5.4%). The 51.5% remainder of the plant species are distributed in 26 families. The bioactive compounds reported here are diverse in structure and, according to their biosynthetic origins, are mainly distributed in terpenoids (30.2%), polyketides (including xanthones, quinones and anthraquinones, 17.3%) and flavonoids (11.3%) classes.

Polyketides (24%) and terpenoids (64%) derived compounds were also the most found in the fungal extracts under study, while from the bioactive bacterial cultures polyketides (66.7%) and fatty acids derivatives (22.2%) were obtained.

From what it has been discussed, we grouped compounds into five groups according to their toxicity to larvae showed by LC_{50} or LC_{90} (expressed in ppm) reported in the papers referenced in the present review: highly active ($0 < LC < 10$), moderately active ($10 \leq LC < 50$), slightly active ($50 \leq LC \leq 100$), very slightly active ($LC > 100$) and inactive (no mortality was observed). From a total of 233 compounds assayed against *Ae. aegypti* larvae 34% of them had an expressive larvicidal effect with LC_{50} or LC_{90} values comprising up to 10ppm. The tiophene derivative α-terthienyl (**26**), the polyacetylene derivative (**30**), the coumarin marmesin (**119**), the flavonoids naringin (**150**), poncirin (**151**) and rhoifolin (**154**), and the amides pellitorine (**279**) guineesine (**280**), pipercide (**281**) and retrofractamide A (**282**) were the most promissors compounds obtained from plants with LC_{50} or LC_{90} below 0.1ppm. Moreover, the two preparation containing avermectins MK-933 (**368**) and MK-936 (**364 + 365**) in addition to spinosad suspension (Tracer®, **369 + 372**) presented LC_{50} values of 0.0234, 0.0104, and 0.025ppm, respectively.

Treatment of larvae with α-terthienyl (**26**) and the furanoacetylene (**30**) exhibited an exceptional phototoxic effect against fourth instar larvae and exceeded the effectiveness of DDT and malathion encouraging the indication of **26** to be a practical larvicide under field conditions [50]. Spinosad proved to be useful in integrated vector management programs targeted at *Ae. aegypti*. Formulations containing this toxin were officially approved for use as a mosquito larvicide by US EPA (United States Environmental Protection Agency) in 2007 and 2008 [264].

Mosquito repellents may be one of the most effective tools for protecting humans from vector-borne diseases.

Although several species of entomopathogenic fungi have currently been considered for use in the biological control of mosquito larvae, very little progress focussing the isolation of bioactive metabolites, have been reported in the literature, especially against *Ae. aegypti*. It has been known that these microorganisms can be possible candidates for use as biological control agents due to their ability to secret bioactive metabolites when they infect their insect hosts. Thus, this source could be more effectively explored as natural resource of insecticides.

ACKNOWLEDGEMENTS

The authors would like to thank Manuela Oliveira de Souza for the helpful assistance with references and Dr. Marcos Malta (UFBA) for the proof-reading.

ABBREVIATIONS

μM	micromolar
1-D NMR	one-dimensional nuclear magnetic resonance
2-D NMR	two-dimensional nuclear magnetic resonance
^{1}H NMR	proton nuclear magnetic resonance
^{13}C NMR	carbon-13 nuclear magnetic resonance
BTI	*Bacillus thuringiensis israelensis* H-14
CNSL	cashew nut shell liquid
CoA	coenzyme A
DDT	dichlorodiphenyltrichloroethane
DEET	*N*,*N*-diethyl-*m*-toluamide
DENV-1	serogroup of dengue virus "dengue-1"
DENV-2	serogroup of dengue virus "dengue-2"
DENV-3	serogroup of dengue virus "dengue-3"
DENV-4	serogroup of dengue virus "dengue-4"
DHF	dengue hemorrhagic fever
DSS	dengue shock syndrome
dTDP	deoxythymidine diphosphate
DV	dengue viruses
EC_{50}	half maximal effective concentration
EIMS	electron-impact mass spectrometry
GC/MS	gas-chromatography–mass-spectrometry
GC–CI–MS	gas-chromatography–chemical-ionization–mass-spectrometry
[H]	reduction
INADEQUATE	incredible natural abundance double quantum transfer experiment
IR	infrared
LC_{50}	lethal concentration 50
LC_{90}	lethal concentration 90
LC_{100}	lethal concentration 100
LD_{50}	lethal dose 50 percent
LD_{90}	lethal dose 90 percent
LD_{100}	lethal dose 100 percent or absolute lethal dose
min	minute
MS	mass spectrometry
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NDP	nucleotidyl-phosphate
NMR	nuclear magnetic resonance
NOEDS	nuclear overhauser enhanced differential spectroscopy
NOESY	nuclear overhauser enhancement spectroscopy
[O]	oxidation
PABA	*p*-aminobenzoate
PAL	*L*-phenylalanine ammonia lyase
PK	polyketide
PKS	polyketide synthase
PNBA	*p*-nitrobenzoate

ppm	parts per million
ROESY	Rotating frame overhause effect spectroscopy
SAM	*S*-adenosylmethionine
TLC	thin layer chromatography
TOCSY	total correlation spectroscopy
US EPA	United States Environmental Protection Agency
WHO	World Health Organization
WHO/TDR	World Health Organization Special Program for Research and Training in Tropical Diseases

REFERENCES

[1] K.S. Vinayaka, S.V.P. Kumar, N. Mallikarjun, T.R.P. Kekuda, Drug Invention Today 2 (2010) 102–105.
[2] M.S. Mulla, Chinese J. Entomol. 6 (1991) 93–104.
[3] F.P. Pinheiro, S.J. Corber, World Health Stat. Q 50 (1997) 161–169.
[4] L. Rosen, Med. Trop. 59 (1999) 495–498.
[5] A.F.U. Carvalho, V.M.M. Melo, A.A. Craveiro, M.I.L. Machado, M.B. Bantim, E.F. Rabelo, Mem. I. Oswaldo Cruz 98 (2003) 569–571.
[6] Dengue and Dengue Haemorrhagic Fever . Fact Sheet no. 117. World Health Organization, 2009.
[7] Dengue Haemorrhagic Fever: Diagnosis, Treatment, Prevention and Control, second ed., World Health Organization, Geneva, 1997.
[8] N.G. Gratz, S.B. Halstead, In: S.B. Halstead (Ed.), Dengue (Tropical Medicine: Science and Practice), vol. 5, Imperial College Press, London, 2008, pp. 361–387.
[9] L.G. Copping, J.J. Menn, Pest. Manag. Sci. 56 (2000) 651–676.
[10] P.M. Dewick, Medicinal Natural Products: A Biosynthetic Approach. John Wiley & Sons Ltd., West Sussex, 2001.
[11] E.S.B. Cavalcanti, S.M. Morais, M.A.A. Lima, E.W.P. Santana, Mem. I. Oswaldo Cruz. 99 (2004) 541–544.
[12] D.E. Champagne, O. Koul, M.B. Isman, G.G.E. Scudder, G.H.N. Towers, Phytochemistry 31 (1992) 377–394.
[13] J.A. Findlay, P.E. Penner, J.D. Miller, J. Nat. Prod. 58 (1995) 197–200.
[14] C.L. Schardl, T.D. Phillips, Plant Dis. 81 (1997) 430–438.
[15] K. Sukumar, M.J. Perich, L.R. Boobar, J. Am. Mosquito Contr. 7 (1991) 210–237.
[16] E.A.S. Shaalan, D. Canyon, M.W.F. Younes, H. Abdel-Wahab, A.H. Mansour, Environ. Int. 31 (2005) 1149–1166.
[17] W.S. Garcez, F.R. Garcez, L. Silva, L. Hamerski, Bioresource Technol. 100 (2009) 6647–6650.
[18] S.A. Fallatah, E.I. Khater, J. Egypt. Soc. Parasitol. 40 (2010) 1–26.
[19] F. Bakkali, S. Averbeck, D. Averbeck, M. Waomar, Food Chem. Toxicol. 46 (2008) 446–475.
[20] B. Adorjan, G. Buchbauer, Flavour Frag. J. 25 (2010) 407–426.
[21] Castro, L.C. Jr., Silva, I.G., Silva, H.H.G. , Rev. Patol. Trop. in press.
[22] S.B. Halstead, In: S.B. Halstead (Ed.), Dengue (Tropical Medicine: Science and Practice), vol. 5, Imperial College Press, London, 2008, pp. 1–28.
[23] D.W.C. Beasley, A.D.T. Barrett, In: S.B. Halstead (Ed.), Dengue (Tropical Medicine: Science and Practice), vol. 5, Imperial College Press, London, 2008, pp. 29–73.
[24] World Health Organization, http://www.who.int/vaccine_research/diseases/vector/en/index1.html [accessed 04/03/2011].

[25] D.J. Gluber (Ed.), New Treatment Strategies for Dengue and other Flaviviral Diseases; Novartis Foundation, John Wiley & Sons, Ltd., Chichester, 2006, pp. 3–22.
[26] B. Guy, F. Guirakhoo, V. Barban, S. Higgs, T. Monath, J. Lang, Vaccine 28 (2010) 632–649.
[27] www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?lvl=0&id=7159 [accessed at 05/03/2011].
[28] H.H. Yap, N.L. Chong, A.E.S. Foo, C.Y. Lee, Kaohsiung J. Med. Sci. 10 (1994) S102–S108.
[29] www.denguevirusnet.com/life-cycle-of-aedes-aegypti.html [accessed 14/02/2011].
[30] O.P. Foratini, Culicidologia Médica, Identificação, Biologia, Epidemiologia, vol. 2, EDUSP, São Paulo, 2002. pp. 860.
[31] C.J. McMeniman, G.L. Hughes, S.L. O'Neill, J. Med. Entomol. 48 (2011) 76–84.
[32] S. Akov, Biol. Bull, Woods Hole 129 (1965) 439–453.
[33] H.H.G. Silva, I.G. Silva, Rev. Soc. Bras. Med. Tro. 32 (1999) 349–355.
[34] W. Arruda, G.M.C. Oliveira, I.G. Silva, Rev. Soc. Bras. Med. Tro. 36 (2003) 17–25.
[35] C.F. Barreto, G.M. Cavasin, H.H.G. Silva, I.G. Silva, Rev. Patol. Trop 35 (2006) 37–57.
[36] R.A. Abed, G.M. Cavasin, H.H.G. Silva, I.G. Silva, Rev. Patol. Trop. 36 (2007) 87–95.
[37] W. Arruda, G.M. Cavasin, I.G. Silva, Rev. Patol. Trop. 37 (2008) 255–267.
[38] R.A. Borges, G.M. Cavasin, I.G. Silva, W. Arruda, E.S.F. Oliveira, H.H.G. Silva, F. Martins, Rev. Patol. Trop. 33 (2004) 91–104.
[39] R.S. Ramsewak, M.G. Nair, S. Murugesan, W.J. Mattson, J. Zasada, J. Agr. Food Chem. 49 (2001) 5852–5856.
[40] A.A. Rahuman, P. Venkatesan, G. Gopalakrishnan, Parasitol. Res. 103 (2008) 1383–1390.
[41] D.H. Kim, S.I. Kim, K.S. Chang, Y.J. Ahn, J. Agr. Food Chem. 50 (2002) 6993–6996.
[42] F. Cepleanu, K. Ohtani, M. Hambruger, M.P. Gupta, P. Solis, K. Hostettman, Helv. Chim. Acta 76 (1993) 1379–1388.
[43] Q. Ye, K. He, N.H. Oberlies, L. Zeng, G. Shi, D. Evert, J.L. McLaughlin, J. Med. Chem. 39 (1996) 1790–1796.
[44] J.K. Rupprecht, C.J. Chang, J.M. Cassady, J.L. McLaughlin, K.L. Mikolajczak, D. Weisleder, Heterocycles 24 (1986) 1197–1201.
[45] T.G. McCloud, D.L. Smith, C.J. Chang, J.M. Cassady, Experientia 43, 1987. 957–949.
[46] S.H. Ho, J. Wang, K.Y. Sim, G.C.L. Ee, Z. Imiyabir, K.F. Yap, K. Shaari, S.H. Goh, Phytochemistry 62 (2003) 1121–1124.
[47] J.K. Rupprecht, Y.-H. Hui, J.L. McLaughlin, J. Nat. Prod. 53 (1990) 237–278.
[48] A.R. Gallimore, Nat. Prod. Rep. 26 (2009) 266–280.
[49] C.K. Wat, S.K. Prasad, E.A. Graham, S. Partington, T. Arnason, G.H.N. Towers, J. Lam, Biochem. Syst. Ecol. 9 (1981) 59–62.
[50] T. Arnason, T. Swain, C.K. Wat, E.A. Graham, S. Partington, G.H.N. Towers, J. Lam, Biochem. Syst. Ecol. 9 (1981) 63–68.
[51] L. Zechmeister, J.W. Sease, J. Am. Chem. Soc. 69 (1947) 273–275.
[52] M.J. Perich, C. Wells, W. Bertsch, K.E. Tredway, J. Am. Mosquito Contr. 11 (1995) 307–310.
[53] M. Nivsarkar, G.P. Kumar, M. Laloraya, M.M. Laloraya, Biochem. Syst. Ecol. 21 (1993) 441–447.
[54] M. Nivsarkar, B. Cherian, H. Padh, Curr. Sci. India 81 (2001) 667–672.
[55] K.E. Schulte, G. Henke, G. Rücker, S. Foerster, Tetrahedron 24 (1968) 1899–1903.
[56] G.C.L. Ee, K.H. Phong, X.H. Mong, K. Shaari, M.A. Sukari, Nat. Prod. Sci. 9 (2003) 174–176.
[57] G.C.L. Ee, A.S.M. Kua, Y.L. Cheow, C.K. Lim, V. Jong, M. Rahmani, Nat. Prod. Sci. 10 (2004) 220–222.
[58] G.C.L. Ee, A.S.M. Kua, C.K. Lim, V. Jong, H.L. Lee, Nat. Prod. Res. 20 (2006) 485–491.

[59] G.C.L. Ee, S. Daud, Y.H. Taufiq-Yap, N.H. Ismail, M. Rahmani, Nat. Prod. Res. 20 (2006) 1067–1073.
[60] T. Sreelatha, A. Hymavathi, J.M. Murthy, P.U. Rani, J.M. Rao, K.S. Babu, Bioorg. Med. Chem. Lett. 20 (2010) 2974–2977.
[61] G. Bringmann, M. Wohlfarth, H. Rischer, M. Rückert, J. Schlauer, Tetrahedron Lett. 39 (1998) 8445–8448.
[62] S.S. Cheng, C.G. Huang, W.J. Chen, Y.H. Kuo, S.T. Chang, Bioresource Technol. 99 (2008) 3617–3622.
[63] A.M.S. Rodrigues, J.E. Paula, F. Roblot, A. Fournet, L.S. Espíndola, Fitoterapia 76 (2005) 755–757.
[64] K.A.L. Ribeiro, C.M. Carvalho, M.T. Molina, E.P. Lima, E. Lopez-Montero, J.R.M. Reys, M.B.F. Oliveira, A.V. Pinto, A.E.G. Santana, M.O.F. Goulart, Acta Trop. 111 (2009) 44–50.
[65] J.R. Ioset, A. Marston, M.P. Gupta, K. Hostettmann, Phytochemistry 47 (1998) 729–734.
[66] J.R. Ioset, A. Marston, M.P. Gupta, K. Hostettmann, Phytochemistry 53 (2000) 613–617.
[67] P. Tiew, J.R. Ioset, U. Kokpol, W. Chavasiri, K. Hostettmann, Phytother. Res. 17 (2003) 190–193.
[68] C.P. Kiprono, J.O. Midiwo, P.K. Kipkemboi, S. Ladogana, Bull. Chem. Soc. Ethiopia 18 (2004) 45–49.
[69] E. Arkoudis, M. Stratakis, J. Org. Chem. 73 (2008) 4484–4490.
[70] G. Bringmann, T.F. Noll, T.A.M. Gulder, M. Grune, M. Dreyer, C. Wilde, F. Pankewitz, M. Hilker, G.D. Payne, A.L. Jones, M. Goodfellow, H.-P. Fiedler, Nat. Chem. Biol. 2 (2006) 429–433.
[71] D.P. Sousa, Y.W. Vieira, M.P. Uliana, M.A. Melo, T.J. Brocksom, S.C.H. Cavalcanti, Parasitol. Res. 107 (2010) 741–745.
[72] Y.C. Yang, M.Y. Lim, H.S. Lee, J. Agr. Food Chem. 51 (2003) 7629–7631.
[73] G.C.L. Ee, Y.P. Wen, M.A. Sukari, R. Go, H.L. Lee, Nat. Prod. Res. 23 (2009) 1322–1329.
[74] I.K. Park, S.C. Shin, C.S. Kim, H.J. Lee, W.S. Choi, Y.J. Ahn, J. Agr. Food Chem. 53 (2005) 969–972.
[75] H. Perumalsamy, K.S. Chang, C. Park, Y.J. Ahn, J. Agr. Food Chem. 58 (2010) 10001–10006.
[76] P.N. Solis, D. Olmedo, N. Nakamura, A.I. Calderon, M. Hattori, M.P. Gupta, Pharm. Biol. 43 (2005) 378–381.
[77] M.M.O. Cabral, J.A. Alencar, A.E. Guimarães, M.J. Kato, J. Am. Mosquito Contr. 25 (2009) 103–105.
[78] E.P. Lichtenstein, T.T. Liang, K.R. Schulz, H.K. Schnoes, G.T. Carter, J. Agr. Food Chem. 22 (1974) 658–664.
[79] A. Marston, K. Hostettmann, J.D. Msonthi, J. Nat. Prod. 58 (1995) 128–130.
[80] J.R. Ioset, A. Marston, M.P. Gupta, K. Hostettmann, J. Nat. Prod. 63 (2000) 424–426.
[81] N.K. Simas, E.D. Lima, S.D. Conceicao, R.M. Kuster, A.M. Oliveira, C.L.S. Lage, Quim. Nova 27 (2004) 46–49.
[82] S.R.L. Santos, V.B. Silva, M.A. Melo, J.D.F. Barbosa, R.L.C. Santos, D.P. Sousa, S.C.H. Cavalcanti, Vector-Borne Zoonot 10 (2010) 1049–1054.
[83] R. Harish, S. Divakar, A. Srivastava, T. Shivanandappa, J. Agr. Food Chem. 53 (2005) 7709–7714.
[84] T. Ozen, I. Demirtas, H. Aksit, Food Chem. 124 (2011) 58–64.
[85] I. Rivero-Cruz, G. Duarte, A. Navarrete, R. Bye, E. Linares, R. Mata, J. Food Sci. 76 (2011) 309–317.
[86] N.S.A. Malik, J.L. Perez, L. Lombardini, R. Cornacchia, L. Cisneros-Zevallos, J. Braford, J. Sci. Food Agr. 89 (2009) 2207–2213.

[87] D.E. Wedge, J.A. Klun, N. Tabanca, B. Demirci, T. Ozek, K.H.C. Baser, Z.J. Liu, S. Zhang, C.L. Cantrell, J. Zhang, J. Agr. Food Chem. 57 (2009) 464–470.
[88] F. Erler, H. Cetin, J. Agr. Urban. Entomol 26 (2009) 31–40.
[89] J. Hussain, N. Bukhari, H. Hussain, N.U. Rehman, S.M. Hussain, Nat. Prod. Commun. 5 (2010) 1785–1786.
[90] B.S. Siddiqui, T. Gulzar, S. Begum, F. Afshan, R. Sultana, Nat. Prod. Res. 22 (2008) 1107–1111.
[91] D.F. Farias, M.G. Cavalheiro, S.M. Viana, G.P.G. Lima, L.C.B. Rocha-Bezerra, N.M.P.S. Ricardo, A.F.U. Carvalho, J. Am. Mosquito Contr. 25 (2009) 386–389.
[92] S. Rajkumar, A. Jebanesan, Parasitol. Res. 104 (2008) 19–25.
[93] P.E.S. De Oliveira, L.M. Conserva, A.C. Brito, R.P.L. Lemos, Pharm. Biol. 43 (2005) 53–57.
[94] A. Oranday, G. Martinez, A. Nunez, C. Rivas, A.E. Flores, Southwest. Entomol. 33 (2008) 315–317.
[95] M. Deshmukh, P. Pawar, M. Joseph, U. Phalgune, R. Kashalkar, N.R. Deshpande, Indian J. Exp. Biol. 46 (2008) 788–792.
[96] J.C. Jung, H.I. Moon, Pharm. Biol. 49 (2011) 190–193.
[97] K. Jewers, J.B. Davis, J. Dougan, A.H. Manchanda, Phytochemistry 11 (1972) 2025–2030.
[98] M. Albuquerque, E.R. Silveira, D. Uchoa, T.L.G. Lemos, E.B. Souza, G.M.P. Santiago, O.D.L. Pessoa, J. Agr. Food Chem. 52 (2004) 6708–6711.
[99] A. Yenesew, J.T. Kiplagat, S. Derese, J.O. Midiwo, J.M. Kabaru, M. Heydenreich, et al., Phytochemistry 67 (2006) 988–991.
[100] J.R. Ioset, A. Marston, M.P. Gupta, K. Hostettmann, J. Nat. Prod. 64 (2001) 710–715.
[101] J.O. Midiwo, A. Yenesew, B.F. Juma, K.L. Omosa, I.L. Omosa, D. Mutisya, 11th NAPRECA Symposium Book of Proceedings, Antananarivo, Madagascar, 2005, pp. 9–19.
[102] A. Yenesew, H. Twinomuhwezi, J.M. Kabaru, H.M. Akala, B.T. Kiremire, M. Heydenreich, M.G. Peter, F.L. Eyase, N.C. Waters, D.S. Walsh, Bull. Chem. Soc. Ethiopia 23 (2009) 409–414.
[103] H.H.G. Silva, I.G. Silva, R.M.G. Santos, E. Rodrigues-Fo., C.N. Elias, Rev. Soc. Bras. Med. Tro. 37 (2004) 396–399.
[104] C.F.B. Valotto, G. Carvasin, H.H.G. Silva, R. Geris, I.G. Silva, Rev. Pat. Trop. 39 (2010) 309–321.
[105] K.V. Rao, S.K. Chattopadhyay, C. Reddy, J. Agr. Food Chem. 38 (1427) (1990) 1430.
[106] M. Hosny, J.P.N. Rosazza, J. Nat. Prod. 62 (1999) 853–858.
[107] G.S. Citoglu, B. Sever, S. Antus, E. Baitz-Gaes, N. Altanlar, Pharm. Biol. 41 (2003) 483–486.
[108] J.E. Irvine, R.H. Freyre, J. Agr. Food Chem. 7 (1959) 106–107.
[109] F. Abe, D.M.X. Donnelly, C. Moretti, J. Polonsky, Phytochemistry 24 (1985) 1071–1076.
[110] A. Yenesew, S. Derese, J.O. Midiwo, M. Heydenreich, M.G. Peter, Pest Manag. Sci. 59 (2003) 1159–1161.
[111] W.D. Ollis, C.A. Rhodes, I.O. Sutherland, Tetrahedron 23 (1967) 4741–4760.
[112] J.N. Vasconcelos, J.Q. Lima, T.L.G. Lemos, M.D.F. Oliveira, M.M.B. Almeida, M. Andrade-Neto, J. Mafezoli, A.M.C. Arriaga, G.M.P. Santiago, R. Braz-Filho, Quim. Nova 32 (2009) 382–386.
[113] L. Crombie, D.A. Whiting, Phytochemistry 49 (1998) 1479–1507.
[114] K. Watanabe, Y. Shono, A. Kakimizu, A. Okada, N. Matsuo, A. Satoh, H. Nishimura, J. Agr. Food Chem. 41 (1993) 2164–2166.
[115] E.C.C. Araujo, E.R. Silveira, M.A.S. Lima, M.A. Neto, I.L. Andrade, M.A.A. Lima, G.M.P. Santiago, A.L.M. Mesquita, J. Agric. Food Chem. 51 (2003) 3760–3762.
[116] Y.S. Jang, J.H. Jeon, H.S. Lee, J. Am. Mosquito Contr. 21 (2005) 400–403.

[117] H. Perumalsamy, N.J. Kim, Y.J. Ahn, J. Med. Entomol. 46 (2009) 1420–1423.
[118] A. Lucia, P.G. Audino, E. Seccacini, S. Licastro, E. Zerba, H. Masuh, J. Am. Mosquito Contr. 23 (2007) 299–303.
[119] G.N. Roth, A. Chandra, M.G. Nair, J. Nat. Prod. 61 (1998) 542–545.
[120] M. Neves, R. Morais, S. Gafner, H. Stoeckli-Evans, K. Hostettmann, Phytochemistry 50 (1999) 967–972.
[121] C.L. Cantrell, J.A. Klun, C.T. Bryson, M. Kobaisy, S.O. Duke, J. Agr. Food Chem. 53 (2005) 5948–5953.
[122] G. Paluch, J. Grodnitzky, L. Bartholomay, J. Coats, J. Agr. Food Chem. 57 (2009) 7618–7625.
[123] C.L. Cantrell, J.W. Pridgeon, F.R. Fronczek, J.J. Becnel, Chem. Biodivers. 7 (2010) 1681–1697.
[124] R. Geris, I.G. Silva, H.H.G. Silva, A. Barison, E. Rodrigues, A.G. Ferreira, Rev. I. Med. Trop. 50 (2008) 25–28.
[125] C.F.B. Valotto, H.H.G. Silva, G. Carvasin, R. Geris, E. Rodrigues-Fo., I.G. Silva, Rev. Soc. Bras. Med. Tro. 44 (2011) 194–200.
[126] M.C. Omena, E.S. Bento, J.E. Paula, A.E.G. Sant'ana, Vector-Borne Zoonot. 6 (2006) 216–222.
[127] A.T.A. Pimenta, G.M.P. Santiago, A.M.C. Arriaga, G.H.A. Menezes, S.B. Bezerra, Rev. Bras. Farmacogn. 16 (2006) 501–505.
[128] M.L. Barreiros, J.P. David, J.M. David, L.M.X. Lopes, M.S. Sa, J.F.O. Costa, M.Z. Almeida, L.P. Queiroz, A.E.G. Sant'Ana, Phytochemistry 68 (2007) 1735–1739.
[129] P. Dzubak, M. Hajduch, D. Vydra, A. Hustova, M. Kvasnica, D. Biedermann, L. Markova, M. Urban, J. Sarek, Nat. Prod. Rep. 23 (2006) 394–411.
[130] C.J. Njoku, L. Zeng, I.U. Asuzu, N.H. Oberlies, J.L. McLaughlin, Int. J. Pharmacogn. 35 (1997) 134–137.
[131] G.M.P. Santiago, F.A. Viana, O.D.L. Pessoa, R.P. Santos, Y.B.M. Pouliquen, A.M.C. Arriaga, M. Andrade-Neto, R. Braz-Filho, Braz, Braz. J. Pharmacogn. 15 (2005) 187–190.
[132] B.P. Chapagain, V. Saharan, Z. Wiesman, Bioresource Technol. 99 (2008) 1165–1168.
[133] S. Siddiqui, S. Faizi, T. Mahmood, B.S. Siddiqui, J. Chem. Soc. Perk. T 1 (1986) 1021–1025.
[134] B.S. Siddiqui, F. Afshan, S. Ghiasuddin Faizi, S.N. Naqvi, R.M. Tariq, J. Chem. Soc. Perk. T 1 (1999) 2367–2370.
[135] B.S. Siddiqui, F. Afshan, S. Ghiasuddin Faizi, S.N.H. Naqvi, R.M. Tariq, Phytochemistry 53 (2000) 371–376.
[136] H. Gurulingappa, V. Tare, P. Pawar, V. Tungikar, Y.R. Jorapur, S. Madhavi, S. Bhat, Chem. Biodivers. 6 (2009) 897–902.
[137] A.K. Kiprop, M.S. Rajab, F.M.E. Wanjala, Bull. Chem. Soc. Ethiopia 19 (2005) 145–148.
[138] A.K. Kiprop, P.C. Kiprono, M.S. Rajab, M.K. Kosgei, Z. Naturforsch. C 62 (2007) 826–828.
[139] A.C. Freitas, M.P. Lima, A.G. Ferreira, W.P. Tadei, A.C.S. Pinto, Quim. Nova 32 (2009) 2068–2072.
[140] M.S. Shivakumar, R. Kataria, Int. J. Pharm. Biosci. 2 (2011) 41–47.
[141] A. Roy, S. Saraf, Biol. Pharm. Bull. 29 (2006) 191–201.
[142] R.S. Ramsewak, M.G. Nair, G.M. Strasburg, D.L. DeWitt, J.L. Nitiss, J. Agr. Food Chem. 47 (1999) 444–447.
[143] E.M.A. Feitosa, A.M.C. Arriaga, G.M.P. Santiago, T.L.G. Lemos, M.C.F. Oliveira, J.N.E. Vasconcelos, J.Q. Lima, G.T. Malcher, R.F. Nascimento, R. Braz, J. Braz. Chem. Soc. 20 (2009) 375–378.
[144] K. Bandara, V. Kumar, U. Jacobsson, L.P. Molleyres, Phytochemistry 54 (2000) 29–32.
[145] A.T. Laranja, A.J. Manzatto, H.E.M.C. Bicudo, Genet. Mol. Biol. 26 (2003) 419–429.

[146] I.K. Park, S.G. Lee, S.C. Shin, J.D. Park, Y.J. Ahn, J. Agr. Food Chem. 50 (2002) 1866–1870.
[147] M. Miyakado, I. Nakayama, H. Yoshioka, N. Nakatani, Agr. Biol. Chem. 43 (1979) 1609–1611.
[148] A. Banerji, D. Bandyopadhyay, M. Sarkar, A.K. Siddhanta, S.C. Pal, S. Ghosh, K. Abraham, J.N. Schoolery, Phytochemistry 24 (1985) 279–284.
[149] Y.C. Yang, S.G. Lee, H.K. Lee, M.K. Kim, S.H. Lee, H.S. Lee, J. Agr. Food Chem. 50 (2002) 3765–3767.
[150] B.S. Siddiqui, T. Gulzar, S. Begum, M. Rasheed, F.A. Saftar, F. Afshan, Helv. Chim. Acta 86 (2003) 2760–2767.
[151] B.S. Siddiqui, T. Gulzar, A. Mahmood, S. Begum, B. Khan, F. Afshan, Chem. Pharm. Bull. 52 (2004) 1349–1352.
[152] B.S. Siddiqui, T. Gulzar, S. Begum, F. Afshan, Nat. Prod. Res. 18 (2004) 473–477.
[153] B.S. Sidiqui, T. Gulzar, S. Begum, Heterocycles 57 (2002) 1653–1658.
[154] B.S. Siddiqui, T. Gulzar, S. Begum, F. Afshan, F.A. Sattar, Helv. Chim. Acta 87 (2004) 660–666.
[155] B.S. Siddiqui, T. Gulzar, S. Begum, F. Afshan, F.A. Sattar, Nat. Prod. Res. 19 (2005) 143–150.
[156] G.M. Strunz, In: Atta-ur-Rahman (Ed.), Studies in Natural Products Chemistry, vol. 24. Elsevier Science B.V., Amsterdam, 2000, pp. 683–738.
[157] C. Nájera, M. Yus, Stud. Nat. Prod. Chem. 21 (2000) 373–455.
[158] K. Watanabe, Y. Takada, N. Matsuo, H. Nishimura, Biosci. Biotech. Bioch. 59 (1995) 1979–1980.
[159] S.E. Drewes, M.M. Horn, J.D. Connolly, B. Bredenkamp, Phytochemistry 47 (1998) 991–996.
[160] S.R. Katade, P.V. Pawar, V.B. Tungikar, A.S. Tambe, K.M. Kalal, R.D. Wakharkar, N.R. Deshpande, Chem. Biodivers. 3 (2006) 49–53.
[161] R. Ratnayake, V. Karunaratne, B.M.R. Bandara, V. Kumar, J.K. MacLeod, P. Simmonds, J. Nat. Prod. 64 (2001) 376–378.
[162] A.A. Rahuman, G. Gopalakrishnan, P. Venkatesan, K. Geetha, A. Bagavan, Phytother. Res. 22 (2008) 1035–1039.
[163] S.K. Madhu, A.K. Shaukath, V.A. Vijayan, Acta Trop. 113 (2010) 7–11.
[164] M.F. Balandrin, S.M. Lee, J.A. Klocke, J. Agr. Food Chem. 36 (1988) 1048–1054.
[165] K. Bandara, V. Kumar, R.C. Saxena, P.K. Ramdas, J. Econ. Entomol. 98 (2005) 1163–1169.
[166] G. Pattersson, Acta Chem. Scand. 18 (1964) 2303–2308.
[167] V.E. Likhovidov, F.S. Isangalin, A.N. Naumov, L.I. Volodina, A.V. Aleksandrova, S.N. Nikolaeva, E.V. Bystrova, E.M. Aslanyan, N.A. Korobova, N.N. Utkina, Russ., 2010, RU 2391389 C2 20100610. (b) V.E. Likhovidov, F.S. Isangalin, A.N. Naumov, L.I. Volodina, A.V. Aleksandrova, S.N. Nikolaeva, E.V. Bystrova, E.M. Aslanyan, N.A. Korobova, N.N. Utkina, Russ., 2010, RU 2391390 C2 20100610.
[168] K.J. van der Merwe, P.S. Steyn, L. Fourie, D.B. Scott, J.J. Theron, Nature 205 (1965) 1112–1113.
[169] J. Varga, S. Kocsubé, Z. Péteri, C. Vágvölgyi, B. Tóth, Toxins 2 (2010) 1718–1750.
[170] J.G. Ondeyka, A.W. Dombrowski, J.P. Polishook, T. Felcetto, W.L. Shoop, Z. Guan, S.B. Singh, J. Indian Biotechnol. 30 (2003) 220–224.
[171] J.G. Ondeyka, A.W. Dombrowski, J.P. Polishook, T. Felcetto, W.L. Shoop, Z. Guan, S.B. Singh, J. Antibiot. 59 (2006) 288–292.
[172] A.G. Medentsev, V.K. Akimenko, Phytochemistry 47 (1998) 935–959.
[173] T.J. Simpson, J. Chem. Soc. Perk. T 1 (1977) 592–595.

[174] J.G. Ondeyka, G.L. Helms, O.D. Hensens, M.A. Goetz, D.L. Zink, A. Taipouras, W.L. Shoop, L. Slayton, A.W. Dombrowski, J.D. Polishook, D.A. Ostlind, N.N. Tsou, R.G. Ball, S.B. Singh, J. Am. Chem. Soc. 119 (1997) 8809–8816.
[175] O.D. Hensens, J.G. Ondeyka, A.W. Dombrowski, D.A. Ostlind, D.L. Zink, Tetrahedron Lett. 40 (1999) 5455–5458.
[176] J.G. Ondeyka, A.M. Dahl-Roshak, J.S. Tkacz, D.L. Zink, M. Zakson-Aiken, W.L. Shoop, M.A. Goetz, S.B. Singh, Bioorg. Med. Chem. Lett. 12 (2002) 2941–2944.
[177] J.G. Ondeyka, K. Byrne, D. Vesey, D.L. Zink, W.L. Shoop, M.A. Goetz, S.B. Singh, J. Nat. Prod. 66 (2003) 121–124.
[178] K.M. Byrne, S.K. Smith, J.G. Ondeyka, J. Am. Chem. Soc. 124 (2002) 7055–7060.
[179] M.M. Smith, V.A. Warren, B.S. Thomas, R.M. Brochu, E.A. Ertel, S. Rohrer, J. Schaeffer, D. Schmatz, B.R. Petuch, Y.S. Tang, P.T. Meinke, G.J. Kaczorowski, C.J. Cohen, Biochemistry-US 39 (2000) 5543–5554.
[180] R.M.G. Santos, E. Roddrigues-Fo, Phytochemistry 61 (2002) 907–912.
[181] R.M.G. Santos, E. Roddrigues-Fo, J. Braz. Chem. Soc. 14 (2003) 722–727.
[182] R.M.G. Santos, E. Roddrigues-Fo, Z. Naturforsch. C 58 (2003) 319–324.
[183] R. Geris, T.J. Simpson, Nat. Prod. Rep. 26 (2009) 1063–1094.
[184] R. Geris, E. Rodrigues-Fo, H.H.G. Silva, I.G. Silva, Chem. Biodivers. 5 (2008) 341–345.
[185] V. Matha, A. Jegorov, J. Weiser, J.S. Pillai, Cytobios 69 (1992) 163–170.
[186] J. Weiser, V. Matha, J. Invertebr. Pathol. 515 (1988) 94–96.
[187] V. Matha, J. Weiser, J. Olejnícek, Folia Parasit. 35 (1988) 379–381.
[188] W.B. Turner, Fungal Metabolites. Academic Press Inc., London, 1971.
[189] B. Siddhardha, U.S.N. Murty, M. Narasimbulu, Y. Venkateswarlu, Indian J. Microbiol. 50 (2010) 225–228.
[190] C. Keller, M. Maillard, J. Keller, K. Hostettmann, Pharm. Biol. 40 (2002) 518–525.
[191] N. Mvoutoulou, J. Invertebr. Pathol. 60 (1992) 208–209.
[192] F. Matsumura, S.G. Knight, J. Econ. Entomol. 60 (1967) 871–872.
[193] F. Washizu, H. Umezawa, N. Sugiyama, J. Antibiot. 7 (1954) 60.
[194] Y. Hirata, H. Nakata, K. Yamada, K. Okuhara, N. Takayuki, Tetrahedron 14 (1961) 252–274.
[195] M.G. Nair, A. Chandra, D.L. Thorogod, Pest Sci. 43 (1995) 361–365.
[196] K. Kakinuma, C.A. Hanson, K.L. Rinehart Jr., Tetrahedron 32 (1976) 217–222.
[197] M.G. Nair, A. Chandra, D.L. Thorogood, J. Antibiot. 46 (1993) 1762–1763.
[198] Y. Ishibashi, S. Nishiyama, S. Yamamura, Chem. Lett. 9 (1994) 1747–1748.
[199] J. He, C. Hertweck, J. Am. Chem. Soc. 126 (2004) 3694–3695.
[200] J. He, M. Müller, C. Hertweck, J. Am. Chem. Soc. 126 (2004) 16,742–16,743.
[201] N. Traitcheva, H. Jenke-Kodama, J.He.E. Dittmann, C. Hertweck, ChemBioChem 8 (2007) 1841–1849.
[202] Y.S. Choi, T.W. Johannes, M. Simurdiak, Z. Shao, H. Lu, H. Zhao, Mol. Biosyst. 6 (2010) 336–338.
[203] M.G. Nair, A.R. Putnam, S.K. Mishra, M.H. Mulks, W.H. Taft, J.E. Keller, J.R. Miller, J. Nat. Prod. 52 (1989) 797–809.
[204] R.W. Burg, B.M. Miller, E.E. Baker, J. Birnbaum, S.A. Currie, R. Hartman, L. Kong, R.L. Monaghan, G. Olson, I. Putter, J.B. Tunac, H. Wallick, E.O. Stapley, R. Oiwa, S. Omura, Antimicrob. Agents Ch. 15 (1979) 361–367.
[205] T.W. Miller, L. Chaiet, D.J. Cole, L.J. Cole, J.E. Flor, R.T. Goegelman, V.P. Gullo, H. Joshua, A.J. Kempf, W.R. Krellwitz, R.L. Monaghan, R.E. Ormond, K.E. Wilson, G. Albers-Schönberg, I. Putter, Antimicrob. Agents Ch. 15 (1979) 368–371.

[206] G. Albers-Schönberg, B.H. Arison, J.C. Chabala, A.W. Douglas, P. Eskola, M.H. Fisher, A. Lusi, H. Mrozik, J.L. Smith, R.L. Tolman, J. Am. Chem. Soc. 103 (1981) 4216–4221.
[207] J.P. Springer, B.H. Arison, J.M. Hirshfield, K. Hoogsteen, J. Am. Chem. Soc. 103 (1981) 4221–4224.
[208] J.A. Lasota, R.A. Dybas, Annu. Rev. Entomol. 36 (1991) 91–117.
[209] W.L. Shoop, H. Mrozik, M.H. Fisher, Vet. Parasitol. 59 (1995) 139–156.
[210] S. Pampiglione, G. Majori, G. Petrangeli, R. Romi, T. Roy. Soc. Trop. Med. H 79 (1985) 797–799.
[211](a) H. Ikeda, S. Omura, J. Antibiot. 48 (1995) 549–562.
(b) H. Ikeda, S. Omura, Chem. Rev. 97 (1997) 2591–2609.
[212] Y.J. Yoon, E.S. Kim, Y.S. Hwang, C.Y. Choi, Appl. Microbiol. Biotechnol. 63 (2004) 626–634.
[213] M.D. Schulman, D. Valentino, M. Nallin, L. Kaplan, Antimicrob. Agents Ch. 29 (1986) 620–624.
[214] H.A. Kirst, K.H. Michel, J.W. Martin, L.C. Creemer, E.H. Chio, R.C. Yao, W.M. Nakasukasa, L.V.D. Boeck, J. Occlowitz, J.W. Paschal, J.B. Deeter, N.D. Jones, G.D. Thompson, Tetrahedron Lett. 32 (1991) 4839–4842.
[215] T.C. Sparks, G.D. Thompson, H.A. Kirst, M.B. Hertlein, J.S. Mynderse, J.R. Turner, T.V. Worden, In: F.R. Hall, J.J. Menn (Eds.), Methods in Biotechnology, vol. 5, (1999), pp. 171–188.
[216] D.R. Hahn, G. Gustafson, C. Waldron, B. Bullard, J.D. Jackson, J. Mitchell, J. Indian Microbiol. Biotechnol. 33 (2006) 94–104.
[217] P. Lewer, D.R. Hahn, L.L. Karr, D.O. Duebelbeis, J.R. Gilbert, G.D. Crouse, T. Worden, T.C. Sparks, P.McK.R. Edwards, P.R. Graupner, Bioorg. Med. Chem. 17 (2009) 4185–4196.
[218] H.A. Kirst, K.H. Michel, J.S. Mynderase, E.H. Chio, R.C. Yao, W.M. Nakasukasa, L.D. Boeck, J. Occlowitz, J.W. Paschal, J.B. Deeter, G.D. Thompson, In: D.R. Baker, J.G. Fenyes, J.J. Steffens (Eds.), Synthesis and Chemistry of Agrochemicals III, vol. 504. American Chemical Society, 1992, pp. 214–225.
[219] K.X. Huang, L. Xia, Y. Zhang, X. Ding, J.A. Zahn, Appl. Microbiol. Biotechnol. 82 (2009) 13–23.
[220] V.L. Salgado, Pestic. Biochem. Phys. 60 (1998) 91–102.
[221] H.A. Kirst, J. Antibiot. 63 (2010) 101–111.
[222] J.G. Bond, C.F. Marina, T. Williams, Med. Vet. Entomol. 18 (2004) 50–56.
[223] C.M. Pérez, C.F. Marina, J.G. Bond, J.C. Rojas, J. Valle, T. Williams, J. Med. Entomol. 44 (2007) 631–638.
[224] G.E. Antonio, D. Sánchez, T. Williams, C. Marina, Pest. Manag. Sci. 65 (2009) 323–326.
[225] C. Waldron, K. Madduri, K. Crawford, D.J. Merlo, P. Treadway, M.C. Broughton, R.H. Baltz, Antonie van Leeuwenhoek 78 (2000) 385–390.
[226] C. Waldron, P. Matsushima, P.R. Rosteck Jr., M.C. Broughton, J. Turner, K. Madduri, K. Crawford, D.J. Merlo, R.H. Baltz, Chem. Biol. 8 (2011) 487–499.
[227] H.J. Kim, R. Pongdee, Q. Wu, L. Hong, H.-W. Liu, J. Am. Chem. Soc. 129 (2007) 14582–14584.
[228] H.J. Kim, J.A. White-Phillip, Y. Ogasawara, N. Shin, E.A. Isiorho, H.-W. Liu, J. Am. Chem. Soc. 132 (2010) 2901–2903.
[229] J. Kiviranta, M. Namikoshi, K. Sivonen, W.R. Evans, W.W. Carmichael, K.L. Rinehart, Toxicon 30 (1992) 1093–1098.
[230] W.W. Carmichael, V. Beasley, D. Bunner, J.N. Eloff, I. Falconer, P. Gorham, K. Harada, T. Krishnamurthy, M. Yu, Toxicon 26 (1988) 971–973.

[231] J. Jizba, G.V. Samoukina, T. Ivanova-Kovacheva, N.V. Kandybin, Folia Microbiol. 37 (1992) 461–462.
[232] K. Harada, M. Suomalainen, H. Uchida, H. Masui, K. Ohmura, J. Kiviranta, et al., Environ. Toxicol. 15 (2000) 114–119.
[233] J. Kiviranta, A. Abdel-Hameed, World J. Microbiol. Biotech. 10 (1994) 517–520.
[234] D.R. Rao, C. Thangavel, L. Kabilan, S. Suguna, T.R. Mani, S. Shanmugasundaram, T. Roy. Soc. Trop. Med. H 93 (1999) 232.
[235] M.G. Murty, G. Srinivas, V. Sekar, J. Inv. Pathol. 64 (1994) 68–70.
[236] S. Kathirgamanathar, W.D. Ratnasooriya, P. Baekstrom, R.J. Andersen, V. Karunaratne, Pharm. Biol. 44 (2006) 217–220.
[237] R.R. Bomfim, A.A.S. Araújo, S. Cuadros-Orellana, M.G.D. Melo, L.J. Quintans-Júnior, S.C.H. Cavalcanti, Lat. Am. J. Pharm. 28 (2009) 580–584.
[238] K.S. Vinayaka, S.V.P. Kumar, N. Mallikarjun, T.R.P. Kekuda, Drug Invention Today 2 (2010) 102–105.
[239] J.V. Rao, P.K. Usman, J.B. Kumar, Afr. J. Biotechnol. 7 (2008) 109–113.
[240] P.K. Mittal, J. Vector Dis. 40 (2003) 20–32.
[241] S. Haq, R.M. Bhatt, K.G. Vaishnav, R.S. Yadav, J. Vector Dis. 41 (2004) 61–66.
[242] H.W. Park, D.K. Bideshi, B.A. Federici, J. Asia-Pacific Entomol. 13 (2010) 159–168.
[243] B.A. Federici, J. Invertebr. Pathol. 89 (2005) 30–38.
[244] K. Gunasekaran, P.S. Boopathi Doss, K. Vaidyanathan, Acta Trop. 92 (2004) 109–118.
[245] L. Regis, S.B. Silva, M.A.V. Melo-Santos, Mem. I. Oswaldo Cruz. 95 (2000) 207–210.
[246] E.J. Scholte, B.G.J. Knols, R.A. Samson, W. Takken, J. Insect. Sci. 4 (2004) 19–42.
[247] J.C. Lord, J. Invertebr. Pathol. 89 (2005) 19–29.
[248] M.R. Faria, S.P. Wraight, Biol. Control 43 (2007) 237–256.
[249] I. Molnár, D.M. Gibson, S.B. Krasnoff, Nat. Prod. Rep. 27 (2010) 1241–1275.
[250] E.J. Scholte, W. Takken, B.G.J. Knols, Acta Trop. 102 (2007) 151–158.
[251] M. Isaka, P. Kittakoop, K. Kirtikara, N. Hywel-Jones, Y. Thebtaranonth, Acc. Chem. Res. 38 (2005) 813–823.
[252] R.O. Silva, H.H.G. Silva, C. Luz, Rev. Pat. Trop. 33 (2004) 207–216.
[253] C. Luz, M.M.H. Tai, A.H. Santos, L.F.N. Rocha, D.A.S. Albernaz, H.H.G. Silva, J. Med. Entomol. 44 (2007) 799–804.
[254] R.N. Leles, N.A. Souza, L.F.N. Rocha, A.H. Santos, H.H.G. Silva, C. Luz, Parasitol. Res. 107 (2010) 1271–1274.
[255] T.B. Clark, W.R. Kellen, T. Fukuda, J.E. Lindegren, J. Invertebr. Pathol. 11 (1968) 1–7.
[256] G.S. Miranpuri, G.G. Khachatourians, Vet. Parasitol. 37 (1990) 155–162.
[257] G.G. Marten, J.W. Reid, J. Am. Mosquito Contr. 23 (2007) 65–92.
[258] N. Valero, E. Meleán, M. Maldonado, M. Montiel, Y. Larreal, L.M. Espina, Revista Científica, FCV-LUZ 16 (2006) 414–419.
[259] N. Sivagnaname, Dengue Bull. 33 (2009) 148–160.
[260] M.C. Tranchida, A. Maciá, F. Brusa, M.V. Micieli, J.J. García, Biol. Control 49 (2009) 270–276.
[261] E.A.-S. Shaalan, D. Canyon, M.W.F. Younes, H. Abdel-Wahab, A.-H. Mansour, Environ. Int. 31 (2005) 1149–1166.
[262] Technical Guides for Diagnosis, Treatment, Surveillance, Prevention and Control of Dengue Hemorrhagic Fever. World Health Organization, Geneva, 1975.
[263] J.W. Pridgeon, J.J. Becnel, G.C. Clark, K.J.; Linthicum, J. Med. Entomol. 46 (2009) 335–341.
[264] M.B. Hertlein, C. Mayrotas, C. Jousseaume, M. Lysandrou, G.D. Thompson, W. Jany, S.A. Ritchie, J. Am. Mosq. Control Assoc. 26 (2010) 67–87.

Subject Index

A

Abnormal heart rhythms, 173
Abscisic acid (ABA), 137
Abutilon indicum, 4, 19
Acalypha arvensis, 4, 26
Acalypha indica, 4
Acanthus spinosus, 4
Acetoxydehydroaustin, 325
(-)-3β-Acetoxylabdan-8(17)-13- dien-15-oic acid, 308
(3*S*)-6-Acetoxymethylene-3,4-dihydro-3-heptantrienyl-8-hydroxyl-5-methylisocoumarin, 205
20-*O*-Acetyl-[3-*O*-(2'E,4'Z)-decadienoyl]-ingenol, 79
(2*E*)-3-[(3*S*)-5-Acetyl-3,4-dihydro-6-methoxy-1-oxo-1*H*-2-benzopyran-3-yl]-2-propenoic acid, 204
6α-*O*-Acetyl-7-deacetylnimocinol, 309
(2*E*,4*E*)-5-[(3*S*)-5-Acetyl-8-(β-D-glucopyranosyloxy)-3,4-dihydro-6-hydroxy-1-oxo-1*H*-2-benzopyran-3-yl] penta-2,4-dienal, 204
Acetylcholine, 32
Acetyl-CoA, 283
8-Acetylsalonitenolide, 302
10β-O-Acetyltransferase, 156
Acetyltriflocusolide lactone, 306
Acetyl-β-D-glucopyranosyl)oxy]-3-[(1*E*,3*E*,5*E*)-hepta-1,3,5-trienyl]- (3*S*)-8-[(6-*O*-3,4-dihydro-6-methoxy-5-methyl-1*H*-2-benzopyran-1-one, 204
AChE inhibition assay, 210
Achillea millefolium, 4, 26, 31, 75, 182
Achyranthes aspera, 4, 26
Achyranthes bidentata, 4
Acorus gramineus, 88
Actinodaphnine, 171
Actinomycete, 279
Activated phagocytes, 263
Adaptogenic agent, 138, 155
Adathoda vasica, 249
S-Adenosylmethionine, 303
Adhesion molecules expression, 128
Adipose tissue, 56
Adonis vernalis, 4, 26
ADR-induced apoptosis, 125
ADR-induced JNK, 126
Aedes aegypti, 277, 281
Aedes larvae, 293
Aedes mosquito, 295
Aerva javanica, 4
Aerva lanata, 4, 25, 26
Aesculus hippocastanum, 26, 32
Aflatoxins, 327
Agaricus xanthoderma, 326
Agathosma betulina, 4
ent-Agatic acid, 308
Ageratum conyzoides, 95
Ageratum sp., 95
Age-related macular degeneration, 163
Agrimonia eupatoria, 4
Agrimonia pilosa Ledeb, 196
Agrimonolide, 196
Agrimonolide 6-*O*-b-D-glucopyranoside, 196
Agropyron repens, 3, 4
Air fresheners, 144
Akt activation, 127
Alantolactone, 77, 307
Albatrellus confluens, 326
Alberta magna, 320
Alcea rosea, 4
Alchemilla arvensis, 4
Alchemilla xanthochlora, 4
Aldehyde-thiol adducts, 185
ent-Alepterolic acid, 308
Algae, 279
Algicidal, 207, 209
Alibertia macrophylla K. Schum, 208
Alisma plantago-aquatica, 4
Alizarin, 291
Alkaloids, 279
Alkylene resorcinols, 92
[6]-Alkylene resorcinols, 92
Allergen, 211
Allicin, 106
Allium ampeloprasum, 4
Allium cepa, 4, 26, 30
Allium sativum, 4, 26
Allium fistulosum var. *caespitosum*, 106

Allium grayi, 68, 86, 106
Allosteric antagonist site, 201
All-*trans*-lycopene, 164
Allyglucosinolate, 102
Allyl isothiocyanate, 100, 101
Allylic hydroxylation, 146
Allylic oxidation, 146
Allylpropyl disulfide, 106
Allylpyrocatechol, 88
Allypyrocatechol diacetate, 88
Almonds *(Prunus dulcis)*, 104
Aloe ferox Miller, 194
Alopecia, 46
Aloperine, 97
Alpha-tocopherol, 125
Alpine liqueur genepy, 47
Alternaria maritima, 209
Alternathera achyrantha, 4
Alzheimer's disease, 259
Amaranthus dubius, 4
Amaranthus hypochondriacus, 4
Amastigotes, 177
Amenorrhoea, 47
γ-Aminobutyric acid receptor (GABAR), 84
Ammi majus, 4
Ammi visnaga, 4
Amodiaquine, 149
Amomum thyrsoideum, 4
Amoscanate, 100
Amphotericin B, 210
Amyotropic lateral sclerosis, 259
α-Amyrin synthase (αAS), 160
β-Amyrin synthase (βAS), 160, 161
Anacardiaceae, 293
Anacardium occidentale, 293
Anaerobic archaea, 266
Anagallis arvensis, 5
Anagyrine, 97
Analgesics, 45
Ancylostoma, 100
Anemone pulsatilla, 5
Anethum graveolen, 5, 31, 187
Anethum graveolus L, 293, 303
Angelica archangelica, 5
Angelica pubescens, 70
Angelica sinensis, 320
Angiogenesis, 59, 118, 126
Anguina tritici, 75
Anisakis sp. larvae, 92
Annona densicoma, 283
Annona purpurea L, 282
Annona squamosa, 5
Annonaceae, 73, 296, 314
Annonaceous acetogenins, 73, 282
Annonacin, 73
Annulatomarin, 201
Anopheles, 52
Anopheles gambiae, 339
Anopheles stephensi, 284
Anthelmintic, 44, 45, 47, 48
Anthocleista nobilis, 5
Anthranilic acid-derived, 100
Anthraquinones, 175, 291
Anthriscus cerefolium, 5, 26
Antiallergic activities, 44, 192
Antiallergic effects, 211
Antiallergy, 44
Anti-angiogenic activity, 122
Anti-apoptotic protein bcl-2 level, 117
Anti-atherogenic effect, 128
Antibacterial, 47-48, 207
Antibiotic-resistant, 55
Anticancer, 84, 192
Anti-cancer (breast), 139
Anti-cancer (colon and lung), 143
Anti-cancer (hepatic), 140
Anti-cancer (leukemia, lung), 140
Anti-cancer (prostate), 143
Anticarcenogenesis activities, 116, 121, 125
Anticatarrhal agent, 48
Anti-chagastic agents, 174
Anticholesterolemic, 48
Anticoagulant, 44, 46, 295
Anti-diabetic, 141, 155
Antidiarrhoeal remedy, 47
Antidiarrhoeic, 48
Antidysenteric, 196
Antiepileptic, 46
Antifeedant, 84, 239, 278
Antifertility agent, 45
Anti-fungal, 178
Antigen-induced degranulations, 211
Antigens, 175
Antihemorragic, 44
Anti-HIV agents, 150
Antihypertension, 44
Anti-inflammatory, 3, 45, 47, 121, 139, 269
Anti-ischemic, 269
Antileukaemic (HL-60) effects, 183
Anti-lipoperoxidant, 269
Antilithi, 3
Antimalarial, 44, 47, 84, 178, 192, 196, 210
Antimalarial medicines, 52
Antimicrobial, 3, 178, 192, 209, 314
Antimutagenic activities, 121
Antimutagenic potential, 126

Antinematodal activity, 68
Antinematodal coumarins, 95
Antinematodal flavonoids, 93
Antinematodal phytochemicals, 108
Antineoplastic activities, 58
Antioxidant, 44, 123, 127, 143, 164, 192, 262
Anti-oxidant (ie. macular degeneration), 143
Antioxidant additives, 274
Antioxidative, 121
Antiparasitic agents, 84, 174, 322
Anti-platelet, 269
Anti-proliferative, 119–120
Antipyretic, 44, 47, 48
Antispasmodic action, 44, 47
Antitoxic, 44
Anti-trypanocidal, 175
Anti-trypanosoma, 186
Antitumora, 309
Antitumoral activities, 44, 47, 59, 178
Antitumoral potential, 211
Anti-tumour efficacy, 126
Antitumour profiles, 61
Antitussive, 46
Antiulcerogenic, 44, 309
Anti-viral (ie. HIV-1), 140, 142
Apaf-1, 118
Aphanes arvensis, 5
Aphelenchoides besseyi, 68, 70, 79
Apiaceae, 303, 320
Apicomplexan parasites, 54
Apigenin, 126–127, 129, 175, 235
Apiofuranosyl-(1→6)-β-D-glucopyranosides, 194
Apiol, 293
Apiospora montagnei Sacc, 203
Apium graveolens, 5, 25, 26, 91, 96
Apocynum androsaemilfolium, 5
Apocynum cannabinum, 5, 26
Apoptosis, 59, 118
Apoptosis induction, 127
Apoptotic cell death, 117
Apoptotic effects, 122
Apoptotic index, 118
Aporphine alkaloids, 175
Appetizer, 47
Apsinthion, 45
Aquifoliaceae, 314
Arachis hypogaea, 68, 270
Aralia racemosa, 5
Araliaceae, 158
Arboviruses, 279
Arbutin, 252
Arbutin content, 254
Arctium lappa, 5
Arctium minus, 5
Arctostaphylos leaf, 252
Arctostaphylos pungens, 252
Arctostaphylos spp, 252
Arctostaphylos tomentosa, 5
Arctostaphylos uva-ursi, 5, 31, 252
Areca catechu, 5, 32
Argemone mexicana, 5, 25, 26, 68–69
Aristolochiaceae, 292, 293
Arnica sachalinensis, 72
Aromatase, 211
Artemdubolides A-H, 62
Artemisinic alcohol (ArtAlc), 150
Artemether, 52, 53, 149
Artemia salina, 338
Arteminolides A, B, C and D, 49, 50, 57, 61, 62
Artemis annua, 150
Artemisia absinthium L, 45
Artemisia abyssinica Schultz-Bip, 45
Artemisia afra (Jacq. Ex. Willd), 45
Artemisia annua L, 43, 45, 78
Artemisia anomala S. Moore, 46
Artemisia apiacea Hance, 46
Artemisia argyi Levl. et Vant, 46
Artemisia asiatica Nakai, 46
Artemisia brevifolia Wall. ex DC, 46
Artemisia canariensis (Bess.) Lessing, 48
Artemisia capillaris Thunb, 46
Artemisia copa Phil, 46
Artemisia diffusa Krasch. ex Poljak, 46
Artemisia douglasiana Besser, 46
Artemisia dracunculus L, 5, 26, 46–47
Artemisia dubia Wall, 50
Artemisia feddei LEV. et VAN, 47
Artemisia fukudo Makino, 47
Artemisia genipi Weber, 47
Artemisia gorgonum Webb, 47
Artemisia herba-alba Asso, 47
Artemisia iwayomogi Kitamura, 47
Artemisia keiskeana Miq, 47
Artemisia khorassanica Podl, 57
Artemisia ludoviciana Nutt, 47
Artemisia mexicana, 5
Artemisia minor Jacq. ex Bess, 47
Artemisia oliveriana J. May ex DC, 47
Artemisia ordosica Krasch, 47
Artemisia princeps Willd, 47–48
Artemisia rubripes, 48
Artemisia santonicum, 48
Artemisia scoparia Waldst. & Kit, 48
Artemisia sphaerocephala Krasch, 48
Artemisia spicigera C. Koch, 48

Artemisia sylvatica Maxim, 48
Artemisia thuscula Cav, 48
Artemisia umbelliformis Lam, 48
Artemisia vetista Wall, 48
Artemisia vulgaris L, 5, 48
Artemisin, 78
Artemisinic acid (ArtA), 150
Artemisinic aldehyde (ArtAld), 150
Artemisinin, 44, 49, 139, 149
Artemisinin antiproliferative effects, 59
Artemisinin biosynthesis, 150, 154
Artemisinin biosynthetic genes, 153
Artemisinin synthesis, 153
Artemisolide, 49, 57
Artesunate, 52, 60, 149
Arthralgia, 279
Arthritis, 142
Ar-turmerone, 306
Arundo donax, 5, 26
Asaparagus officinalis, 5
Asaparagus racemosus, 5
β-Asarone, 88
α-Asarone, 88, 293
Asarum canadense, 5
Asarum europaeum, 5
Asarum heterotropoides, 292, 293
Asarum sieboldii, 6
Ascaridia galii, 75
Ascaridiasis, 77
Ascaridole, 74
Asclepias curassavica, 6
Ascochin, 206
Ascorbic acid (vitamin C), 267
Asimicin, 73
Asimina longifolia, 282
Asparagus, 106
Asparagus officinalis, 106
Asparagusic acid, 106
Aspergillus flavus, 210
Aspergillus funiculosus, 326
Aspergillus melleus, 201, 325
Aspergillus ochraceus, 203, 321
Aspergillus terreus, 193
Asplenuim scolopendrium, 6
Asteraceae, 49, 201, 284, 295, 296, 307
Asthma, 56
Astringent, 3
Ataxia telangiectasia mutated (ATM), 127
Atherogenesis, 268
Atherosclerosis, 259
ATP-bioluminescence assay, 177
Aurantiogliocladin, 321
Aureothin, 327, 328
Autooxidation, 261
Avencins, 158
Avermectin, 329
Avermectin B1a, 329
Avermectins MK-933, 366
Aynodon dactylon, 6
Azadirachitin, 313
Azadirachtin A,B, 80, 239
Azadirachta indica, 6, 26, 80, 309, 320
Azadiradione, 311
Azoxymethane-induced, 211

B

Bacillus megaterium, 206, 209, 210
Bacillus sphaericus, 339
Bacillus subtilis, 209
Bacillus thuringiensis var. *israelensis*, 278
Bacopa monnieri, 6
Bacterial lipopolysaccharide (LPS), 56
Bacteroides melaninogenicus, 209
Bacticide, 339
Baeyer–Villiger-like oxidation, 325
Balanitaceae, 309
Balanites aegyptiaca, 309
Baliospermum montanum, 6
Bambusa arundiacea, 6
Barosma betulina, 6
Barosma serratifolia, 6
Batis maritima, 6
Bauhinia racemosa, 6
Bayogenin, 81
Bcl-2 activity, 122
Bcl-2 family proteins, 118
Bearberry, 252
Beauveria bassiana, 278, 340
Begonia cucullata, 6
Begonia sanguinea, 6
Benincasa hispida, 6
Benzaldehyde, 75
Benznidazole, 174
Benzocaine, 243
3,4-Benzopirene carcinogenic potency, 119
Benzyl isothiocyanate, 101
Benzylglucosinolate, 102
Berkheya adlamii, 72
Beta-carotene, 162
Betula pendula, 6
Betulinic Acid, 142
BHA, 268
BHT, 268
Bidens pilosa, 6, 26
Bidens tripartita, 6
Bidesmoside saponin, 309

Bioaccessibility, 129
Bioactive sesquiterpene lactones, 43
Bioassay, 340
  against *Aedes aegypti* larvae, 340
Bioassays, 314
Bioautography assay, 210
Biodiversity consequences, 233
Biological activities, 155, 159, 163
  of carotenoids, 163
  of ginsenosides, 159
  of taxol, 155
Biological control, 338
  of *Ae. aegypti*, 338
Biological processes, 260
Biomass growth factors, 158
Biopesticides, 278
Biosynthesis, 144, 150, 160, 164, 328
  of artemisinin, 150
  of aureothin, 328
  of carotenoids, 164
  of ginsenosides, 160
Biosynthetic origin, 324
  of nodulisporic acid, 324
Biosynthetic pathway, 147, 313, 318, 319, 330, 334
  for the amine components of *Piper* amides, 319
  for the straight-chain of *Piper* amides, 318
  of (-)-menthol, 147
  of avermectins, 330
  of spinosyns, 334
  to limonoids, 313
Biosynthetic work, 301
  on the rotenoids, 301
Biotechnological approaches, 144
Biotechnology, 152, 161, 165
1,3-Bisphosphoglycerate (BPG), 177
Bis-xanthone, 322
Bitoscanate, 100
Bixa orellana, 6, 19, 26
Black dead diseases, 207
  of grapevine, 207
Blood-letting, 2
Blumea lacera, 6
Bocconia cordata, 97
Bocconine, 97
Boerhaavia hirsuta, 6, 26
Boerhavia diffusa, 6
Boraginaceae, 289
β-Boraginaceae, 293
Borago officinalis, 6
L-Borneol, 75
Borreria verticillata, 6
Boswellia serrata, 6
Botryosphaeria obtuse, 207
Botrytis cinerea, 55, 212
Bovine viral diarrhoea virus, 55
Brasilixanthone, 285
Brassica napus, 101, 165
Brassica napus cultivars, 102
Brassica nigra, 100
Brassica oleracea, 6, 26, 31
Brassica spp, 100
Breast cancer cell line, 117
Brofifacoum, 295
Bromhexine, 248, 249
Bronchodilator, 46
Brucea mollis var. *tonkinensis*, 84
Brucein B,D, 84
Bruising, 47
Brusatol, 84
Bryophyllum pinnatum, 6, 26
Buddleia americana, 6
Buddleia marrubiifolia, 7
Buddleja crispa, 87
Bullatacin, 282
Bupleurum salicifolium, 91
Bursaphelenchus lignicolus, 70
Bursaphelenchus oxylophilus, 97
Bursaphelenchus xylophilus, 73, 78, 79
Bursera gumifera, 7
5-(But-3-ene-1-ynyl)-2-2´-bithiophene, 284
5-(But-3-ene-1-ynyl)-5´-methyl-2-2´-bithiophene, 284
But-3-enylglucosinolate, 102
Butea frondosa, 75
Butea monosperma, 7
Butein, 272
5-(1-Buten-1-ynyl)-2,2'- bithiophene, 72
Butyl-6,8-dihydroxy-3(*R*)-pentylisochroman-1-one, 203
Butylated hydroxytoluene, 267
12-*n*-Butyldeoxoartemisinin, 150
Tert-Butyldodecadienamide, 316

C

Caenorhabditis elegans, 73, 79, 80, 85, 92, 94, 96, 100, 101
Caesalpinia bonduc, 7
Cafestol, 140
Caffeic acid, 87, 121, 122, 237, 271
4-O-Caffeoyl quinic acid, 271
Cakes products, 232
Caki-I carcinoma, 122
Calcarisporium arbuscula F-80, 322

Calcium ionophore (PMACI)-stimulated mast cells, 128
Calliandria anomala, 7
Callicarpa americana, 306
Callicarpa species, 320
Callicarpenal, 306, 320
Callitris arborea, 7
Callosobruchus maculates, 320
Calmodulin, 122
Calodendrolide, 311
Calodendrum capense thumb, 311
Calophyllum inophyllum, 285
Calystegia soldanella, 7
Camaric acid, 80
Camarin, 80
Camarinin, 80
Camellia sinensis, 7, 26, 30
Camphene, 137
L-Camphor, 75
Camphor, 187
Camponotus herculeanus, 212
Camponotus ligniperda, 212
Camponotus ramulorum, 212
Canarian folklore, 48
Cancer, 124, 259
Cancer (breast), 141
Candida albicans, 187, 203, 210
Candida glabrata, 210
Candidissiol, 137
Canescin, 193
Canna edulis, 7
Canna indica, 7
Canna latifolia, 7
Capriola dactylon, 7
Capsella bursa pastoris, 7, 26
Capsicum annuum, 7, 27, 30
Carassius auratus, 98, 340
5-Carboxymellein, 203
Carbuncle, 47
Carcinogenesis, 58, 123
Carcinogenic properties, 321
Cardenolide N-1, 85
Cardiomyopathy, 141, 173
Cardioprotective, 124
Cardiovascular diseases, 124
δ-3-Carene, 75
Carex arenaria, 7
Carex hirta, 7
Carica papaya, 101
Carlina acaulis, 7
Carminative, 48
Carnosic acid, 129
Carnosol, 129
β-Carotene, 137, 269, 270, 343
ζ-Carotene desaturase, 164
Carotenoid biosynthesis, 164
Carotenoid isomerase, 164
Carotenoids, 137, 144, 161, 261, 273
Carrageenan-induced inflammation phases, 57
Cartesian bi-dimensional world, 257
Carthamus tinctorius, 70
Carum carvi, 75, 187
Carvacrol, 180, 185, 186, 305
d-Carvone, 120
Carvone, 185
Caryocar glabrum (Aubl.) Pers, 199
β-Caryophyllene, 140, 307
Caryophyllene oxide, 75
Caspase, 122
Caspase activation, 118
Caspase-3, 118
Caspase-3-dependent apoptosis, 60
Caspases, 118
Cassava, 105
Cassava *Manihot esculenta*, 104
Cassia fistula, 7, 27
Cassia obovata, 243
Cassia obtusifolia, 291
Cassia occidentalis, 7, 27
Cassia siamea Lam, 193
Cassia tora, 7
Cassia obovata, 243
Cassythine, 175
Catalases, 263, 264, 266
Catechin, 175
Catechin supplementation, 127
Catechins, 124, 127
Catharanthus roseus, 96
Cation channel subfamily M member 8) ion channel, 145
Catunaregam spinosa (Thunb.) Tirveng, 199
Caulophyllum thalictroides, 7, 27
CCL, 11, 129
Cdc, 42, 123
Cecropia peltata, 7, 27
α-Cedrene, 137
Cedronin, 84
Cedrus deodara, 7
Cedrus derodara, 32
Cedrus libani, 7
Cellular antioxidant enzyme systems, 265
Cellular hydrogen peroxide, 266
Cellular signalling pathways, 59
Centaurea cyanus, 7
Centaurium erythraea Rafn, 200
Centella asiatica, 7, 27, 161

Cephalanthus occidentalis, 7
Cephalosporium sp, 208
Cephalotaxus hainanensis Li, 204
Cephalotaxus mannii Hook. f, 206
Cerebral malaria, 149
Cerebrovascular diseases, 274
Ceruloplasmin, 265
Cestrum nocturnum, 8
α-Chaconine, 85
Chagas disease, 173
Chagasic cardiac disease, 173
Chain reactions, 261
Chain-carrying peroxy-radicals, 268
Chalcones, 175
Chamaecyparis obtuse, 303
Chamaelirium luteum, 8
Chamaemelum nobile, 8
Chaparrinone, 84
Chavibetol, 88
Chavibetol acetate, 88
Chavicol, 88
Chelerythrine, 97
Chelidonium majus, 98
Chemical coloring additives, 243
Chemical insecticides, 67, 238
Chemical nematicides, 67
Chenopodiaceae, 181
Cherimolin, 73
Chimaphila umbellata, 8
ChimeriVax technology, 280
Chinese pharmacopoeia, 233
Chiranthoden pentadactylon, 8
Chiricanines A-E, 297
Chitranone, 286
(4*S*)-(+)-*Chlorella fusca*, 206
Chlorella fusca, 209, 210
Chloris distichophylla, 8
7-Chloro-3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin, 202
Chlorogenic acid, 124, 235, 271
2α-Chloro-iso-secotanapartholide, 50
Chlorophylls, 137
Chloroquine, 52
Cholagogue, 48
Choleretic, 47
Chondrodendron tomentosum, 8, 32
Chorioallantoic membrane neovascularisation, 61
Chroman-4-one, 93
Chromanones, 296
Chromolaena odorata, 99
Chromosomal aberrations, 118
Chronic myeloid leukaemia therapy, 61
Chronic myeloid leukaemic K562 cells, 60
Chrysanthemum, 278
Chylomicrons, 268
Cichorium intybus, 8
Cimicifuga racemosa, 8, 27
Cinchona L, 43
Cineole, 75
1,8-Cineole, 185
Cinerariaefolium (Asteraceae), 278
Cinnamaldehyde, 185, 187
Cinnamic acids, 272
Cinnamic aldehyde, 293
Circulatory disorders, 48
Cirsium japonicum, 70
Cisplatin, 59
Cissampelos capaeba, 8
Cissampelos owariensis, 8
Cissampelos pariera, 8, 27
Citral, 75
Citronellal, 75, 137
Citronellol, 75
Citrullus colocynthis, 282
Citrullus lanatus, 8
Citrus aurantium, 8, 27, 32
Citrus decumana, 8
Citrus limonum, 8
Citrus peel oil, 178
Citrus sinensis, 7, 27, 31
Cladosporium sphaerospermum, 210
Claytonia sibirica, 8
Clematis biondiana, 8
Clematis gouriana, 8
Clematis vitalba, 8, 20, 25, 31
Clusiaceae family, 285
Cocculus pendulus, 8
Cochlearia armoracia, 8
Cochlearia officinalis, 8
Cochliobolus miyabeanus, 209
Coenzyme Q, 272
Coffea arabica, 8, 30
Coffea species, 314
Cola acuminata, 8
Cola species, 314
Colchicine, 100
Colds, 48
Colecistitis, 47
Coleoptera, 333
Colletotrichum acutatum, 55
Colletotrichum fragariae, 55
Colletotrichum gloesporioides, 55
Collinsonia canadensis, 8
Collisonal quenching, 260
Colon carcinoma Colo, 205, 62

Colonic aberrant, 211
Colonic mucosal ornithine decarboxilase, 211
Colosolic acid, 175
Combretum micranthum, 8, 27
Commercial phenylpropanoids, 293
Conjugated dienes, 120
Consolida regalis, 9
Contact dermatitis, 211
Convallaria majalis, 9, 27, 32
Convolvulanic acids, 207
Convulsions, 256
Conyza canadensis, 9
Conyza serpentina, 9
Copaifera langsdorffii, 9
Copaifera reticulata Ducke, 308
Copper catalyzed oxidation, 127
Coprophilous fungus, 203
Corchorus olitorius, 9
Cordia alliodora, 293
Cordia linnaei, 289
Cordia salicifolia, 9, 27
Cordiaquinone skeleton, 289
Cordiaquinones B, 289
Coreopsis lanceolata, 70
Corossolin, 73
D-Corydaline, 98
Corydalis turtschaninovii, 98
Costus spicatus, 9
Cotton pellet-induced granuloma, 57
Coumarin marmesin, 366
Coumarins, 95, 279, 294
Coumestrol, 94
COX-2 gene, 118
Crassacephalum crepidioides S. Moore, 203
Crassocephalum biafrae S. Moore, 200, 209
Crataegus mexicana, 9
Crataegus oxyacantha, 9, 27
Crateva religiosa, 9
Crescentia linearifolia, 9
Crithmum maritimum, 9
Crotalaria sp., 99
Crotalaria spectabilis, 99
Croton regelianus, 74
Croton zehntneri var, 183
Cruzipain, 175
Cucumis sativus 9, 25, 27, 31, 33
Cucurbita maxima, 9
Cucurbita pepo, 9
Cucurbitin, 31
Cupressaceae, 303
Curcuma aromatica, 320
Curcuma longa L, 272, 306, 308
Curcumin, 272
Cyanobacteria, 279
Cyanogenic glucosides, 104
Cyanosis, 256
4-Cyclohexyl-3-isopropyl-BPT, 84
Cyclooxygenase, 263, 269
Cyclooxygenase pathway, 128
Cyclooxygenase-2 (COX-2), 56, 117
Cyclophosphamide, 118
Cyclosporin A, 326
Cycochalisines, 207
Cynanchum vincetoxicum, 9
Cynodon dactylon, 9, 27, 33
Cypermethrin, 278
Cyperus rotundus, 9
Cyst hatching, 91
Cysteine, 261
Cystitis, 31
Cytisus scoparius, 9, 27, 33
Cytochrome C, 118
Cytochrome C release, 117, 118
Cytochrome P450 enzyme, 211
Cytochrome P450 monooxygenase, 154
Cytokines, 55, 56
Cytoplasmatic vacuolation, 308
Cytoplasmic blebbing, 119
Cytoskeletal reorganization, 123
Cytosol, 117, 135
Cytospora eucalypticola van der Westhinger, 204
Cytotoxic, 44
Cytotoxic assays, 119

D

Dactylogyrus intermedius, 98
Daidzein, 300, 301
Dammarane and oleanane type ginsenosides, 158
Damnacanthal, 291
Danthron, 291
Daphne odora, 79
Daucus carota, 163
Daucus carota L, 163, 193
7-*O*-Deacetal-23-*O*-methyl-7-*O*-senecioylnimocinolide, 309
10-Deacetylbaccatin, 3, 156, 157
Deacetyllaurebiolide, 57
Deacetylspathelin, 312
3-*O*-(2'*E*,4'*Z*)-Decadienoylingenol, 79
Deguelin, 300, 303
Dehydrases, 265
6a,12a-Dehydroβ-toxicarol, 300
Dehydroascorbate, 268
Dehydroascorbic acid, 268

Dehydroaustin, 325
Dehydrocostus lactone, 175, 306
Dehydrodeguelin, 300
Dehydrogingerdione, 320
Dehydroleucodine, 51, 56
Cis-Dehydromatricaria ester, 70
Delirium, 256
Delphinium staphisagria, 9
Demethylannulatomarin, 201
3-Demethylkigelin, 193
Demulcent, 3
Dendrocalamus strictus, 9
Dengue, 238
Dengue haemorrhagic fever (DHF), 279
Dengue viruses, 279
Dengue-1 (DENV-1), 279
Dengue-2 (DENV-2), 279
Dengue-3 (DENV-3), 279
Dengue-4 (DENV-4), 279
Densitometric approach, 222
DENV serotypes, 280
Deoxoartemisinin trimer, 59
Deoxy xylulose phosphate (DOXP), 176
1-Deoxy-D-xylulose-5-phosphate reductoisomerase (DXR), 147
1´-Deoxy-nodulisporic acids, 322
Derivative α-terthienyl, 366
Dermal Em, 146
Derris, 98
Derris foliate, 300
Derris trifoliate, 297, 298
Desmethylagrimonolide 6-*O*-β-D-glucopyranoside, 196
Desmodium gangeticum, 9
Desulphoferrodixin, 266
Dexamethasone, 119
Dhurrin, 105
Diabetes, 48, 142
Diabetes mellitus, 45, 259
Diallyl disulfide, 320
Diaminotoluenes, 243
Dianthus superbus, 9, 20
Diarrhoea, 48
Diayangambin, 292
Dibenzofuranones, 175
Dicentrine, 175
5,7-Dichloro-3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin, 202
Dicipelium cariophyllatum, 183
Dictamnus albus, 9
Didelta carnosa, 72
Diels–Alder reaction, 289
Difethialone, 295
Digestive, 47
Diginosyl)-14,15α-dihydroxy-5α- 3β-*O*-(β-D-card-20(22)-enolide, 85
Digitalis purpurea, 9, 27
3,4-Dihdroxy-phenylacetic acid, 122
(3*S*)-3,4-Dihydro-3-heptantrienyl-8-hydroxyl-6-hydroxymethyl-5-methylisocoumarin, 205
(3*R*)-3,4-Dihydro-6,8-dihydroxy-3- (2'-acetyl-3',5'-dihydroxyphenyl) methyl-1*H*-[2] benzopyran-1-one, 194
3,4-Dihydro-6,8-dihydroxy-3-(2'-acetyl-3'-hydroxy-5'-methoxy-phenyl)methyl-1*H*-[2]benzopyran-1-one, 194
Dihydroartemisinic acid, 150, 153
Dihydroartemisinin, 52, 59
Dihydroartemisinin 12-benzoate, 59
11-α-H-Dihydrodehydrocostus lactone, 306
3,3,-*O*-Dihydrogedunine, 311
6-Dihydrogingerdione, 320
Dihydroguaiaretic acid, 90
Dihydroinversin, 193
Dihydroisocoumarin, 199, 204
Dihydrolapachol, 287
3-n-Butyl-4,5-dihydrophthalide, 91
Dihydroquercetin, 270
2β,3β-Dihydroxy-23-oxo- olean-12-en-28-oic acid, 81
(3*R*)-6,8-Dihydroxy-3-(6-hydroxyundecyl)-3,4-dihydroisocoumarin, 196
Dihydroxy-3-(6-oxoundecyl)-3,4-(3*R*)-6,8-dihydroisocoumarin, 196
7-But-15-enyl-6,8-dihydroxy-3(*R*)-pent-11-enylisochroman-1-one, 203
(3*R*)-6,8-Dihydroxy-3-undecyl-3,4-dihydroisocoumarin, 196
1β,6α-Dihydroxy-4(15)-eudesmene, 51
2´-6-Dihydroxy-4´-methoxychalcone, 298
1α,4β-Dihydroxy-8α-acetoxy-guaia-2,10(14),11(13)-triene-6,12- olide, 51
Dihydroxyflavone, 271
Cis-4,6-Dihydroxymellein, 203
(3*R,4R*)-4,7-Dihydroxymellein, 207
Dihydroxyphenyl lactic acid, 125
6α,7β-Dihydroxyvouacapan-17β- oic acid, 309
Dill greens, 303
Dill-apiol, 293
Dimeric artemisinins, 60
Dimeric DHIC tragoponol, 197
Dimeric guaianolides, 50, 57, 62
Dimethl trisulfide, 106
2,3-Dimethoxy 5,6-dimethylbenzoquinone, 321

6,7-Dimethoxy-4-chromanone, 297
(3*R*,4*R*)-6,7-Dimethoxy-4-hydroxymellein, 202
4-(3´,4´-Dimethoxyphenyl)buta-1,3-diene, 320
3,6-Dimethyl-2-hydroxy-4-methoxybenzoic acid, 337
2,2-Dimethyl-6-vinylchroman-4-one, 297
3,5-Dimethyl-8-methoxy-3,4-dihydroisocoumarin, 205
Dimethylallyl diphosphate (DMAP), 176
Dimethylbenz[a]anthracene-induced (DMBA) DNA adduct formation, 117
2,5-Dimethyl-*para*-benzoquinone, 290
2,6-Dimethyl-*para*-benzoquinone, 290
Dioscorea cayenensis, 9
Dioscorea deltoidea, 85
Dioscorea nipponica, 197
Dioscorea villosa, 10
Diosphenol, 311
Diphenyl propanes, 269
Diplolophium buchanani, 293, 294
Diplonychus indicus, 340
Dipropyl disulfide, 106
Dipsacus fullonum, 10
Diptera, 333
Dirca palustris, 282
Diterpenes, 175
Ditylenchus destructor, 77
Ditylenchus dipsaci, 96
Diuresis, 44
Diuresis-inducing chemicals, 30
Diuresis-inducing phytochemicals, 35
Diuretic, 3, 47, 141, 155
Di-*n*-Propyl disulfide, 320
Di-radical character, 261
DMBA detoxification enzymes, 117
DMBA-activating enzymes, 117
DMBA-induced mammary carcinogenesis, 123
DMBA-induced mouse skin, 123
DMBA-induced skin carcinogenesis, 123
DMBA-induced tumourigenesis, 119
DMDP, 99
DNA damage, 58
[(2*E*,4*E*)-Dodecadienoyl]-**N**-sobutylamide, 315
[(2*E*,4*E*)-Dodecadienoyl]-pyrrolidine, 315
Dolabellane-1, 140
Dorstenia brasiliensis, 10
Dorycnium pentaphyllum 87
Doxorubicin, 120, 126
DPPH method, 274
DPPH radical, 274
DR5 up-regulation, 126
Dracocephalone A, 175
Dracocephalum kotschyi, 179
Drimia indica, 10
dRLh-84 (rat hepatoma), 122
Drosophila melanogaster, 324
α-Duprezianene, 137
DXR mutagenicity, 126
Dysentery, 47
Dysfunction, 247
Dyslipidemia, 120
Dyspepsia, 248

E

Eclipta prostrata, 72
Eczema, 46, 48, 211
Eflornithine, 174
Egg maturation, 280
Eimeria tenella, 55
Elemicin, 293
Elicitors, 158
Elymus repens, 10
Embelia ribes, 10
Embelia schimperi, 289
Emmenagogue, 48, 141, 155
Emodin, 291
Endoperoxide, 49
Endoperoxide bridge structure, 53
Endoperoxide sesquiterpene, 148
Endophytic fungi, 148
Endophytic *Pezicula* species, 209
Endoplasmic reticulum-stress induction, 60
Endothelial cell mitogen, 60
Enhanced taxol production, 158
Enlarged esophagus, 173
Entomopathogens, 338
Enzyme inhibition, 262
Eosinophil recruitment, 129
Epicatechin, 175
Epidermophyton floccosum, 209
Epigallocatechin, 175
Epilachna paenulata, 55
Epileptiform convulsions, 77
Epimastigotes, 177, 178, 183
Epoxy-*β*-farnesene, 289
Epoxyazadiradione, 311
1α,2α-Epoxynimbocinol, 311
Epoxynimolicinol, 311
3α,4α-Epoxyrupicolins C-E, 57, 58
Equisetum arvense, 3, 10, 25, 27, 237
Equisetum bogotense, 10, 27
Equisetum raw materials, 237
Equisetum robustum, 10

Eragrostis curvula, 86
Eremophilanolides, 49
Erigeron philadelphicus, 70
Eriodictyon californicum, 10
ERK activation, 119
Eryngium campestre, 10
Eryngium comosum, 10
Eryngium maritimum, 10
Eryngium planum, 10, 21
Eryngium yuccifolium, 10
Erysimum cheiri, 10, 21
Erythritol phosphate (MEP or nonmevalonate) pathway, 176
Erythrocentaurin, 200
Erythroleukemic (K-562) cell lines, 122
Erythrophleum guineense, 10, 33
Erythroxylaceae, 309
Erythroxylum passerinum, 309
Escherichia coli, 209, 210
Esenbeckia grandiflora, 295
Espintanol, 180, 186
Estragole, 75
Ethyl gallate, 87
12-(2'-Ethylthio) deoxoartemisinin dimer, 59
Etoposide, 43
EU market, 218
  for food supplement, 218
Eucalyptus camadulensis, 303
Eucalyptus meliodora, 75
Eucerin, 146
Eudesmanolides, 44, 49, 51, 307
γ-Eudesmol, 75–76
10-*epi*-γ-Eudesmol, 307
Eugenol, 75, 117, 183, 293
Eugenoliferum, 183
Eupatorium, 296
Eupatorium purpureum, 3, 10
Eupatorium cannabinum, 10
Eupatorium perfoliatum, 10
Euphorbia cyparissias, 10
Euphorbia hyssopifolia, 10
Euphorbia kansui, 79
Eurotium repens, 209
Evodia rutaecarpa, 99
Excited state, 260
Exogenous menthol, 148
Expectorants, 2, 48
Expression, 117
Extractible hydrogen atoms, 261
Extracts, 229
  of passion flower, 229–230
Eysenhardtia polystachya, 10

F

Fabaceae, 291, 293, 297, 306
Fabiana imbricate, 152
Faeriefungin, 329
Faeriefungin A,B, 329
Family Flaviridae, 279
β-Farnesene, 289
Farnesol, 140
E,E-Farnesol, 306
Farnesyl diphosphate (FPP), 135
Farnesyl diphosphate synthase, 161
Farnesyl-protein transferase (FPTase), 61
Fecundity suppression, 238
(+)-Fenchone, 303
Fenitrothion, 278
Fenthion, 278
Feralolide, 194
Ferruginol, 79
Ferulic acid, 121, 122
Fever, 46, 47, 279
Filariasis, 238
Filipendula ulmaria, 10, 27
Fingerprints of *Lawsonia inermis*, 244
Fisetin, 129, 270
Fish predators, 340
Flavanols, 124
Flavanones, 124, 269
Flaveria repanda, 72
Flavivirus genus, 279
Flavoenzymes, 263
Flavone glycosides, 93
Flavones, 124
Flavonoids, 124, 256, 262, 269, 279
Flavonols, 124
Flax *Linum usitatissimum*, 104
Cis-Flocoumafen, 295
5-Fluorouracil, 59
Foeniculum vulgare, 10, 27, 30, 75
Formononetin, 303
2-Formyl-1-hydroxyanthraquinone, 291
5-Formyl-8-hydroxy-3-methyl-3,4-dihydroisocoumarin, 205
Forskolin, 141
Fourth instar mosquito larvae, 294
FPTase inhibitors, 61
Fragaria vesca, 10
Fraxinus excelsior, 10
Free radical chemistry, 259
Free radical oxidation, 120
Free radical scavenger, 262
Freund's adjuvant carrageenan, 57
Frulic acid, 87
Fumaria officinalis, 11

Fungal elicitor, 158
Fungal endophytes, 146
Fungal entomopathogens, 339
Fungal infected carrots, 202
Fungicidal, 207, 209
Funtumia africana, 11, 27
Furanocoumarins, 294
Furfural, 73
Furosemid, 35
Fusamarin, 202
Fusarium oxysporum, 209
Fusarium solani, 210
Fusarium splendens, 209
Fusobacterium nucleatum, 209

G

G protein isoprenylation, 120
G1 cell cycle arrest, 59
$GABA_A$ receptors, 201
Gaeumannomyces graminis, 158
Gaillardia pinnatifida, 11
Gaillardia pulchella, 72
Galega officinalis, 11
Galium aparine, 11
Galium verum, 11, 22
Gall bladder inflammation, 48
Gallic acid, 87, 122
Gallocatechin, 175
Garcinia cuneifolia, 285
Garcinia mangostana, 286
Gastric cancer, 56, 120
Gastrointestinal disease, 46, 173
Gaultheria procumbens, 11, 27
Geanial, 185
Gedunin, 311
Gemcitabine, 126–127
Gemmo derivatives, 251
Genista tinctoria, 11, 27
Genistein, 300
Genitourinary, 3
Genotoxic mechanism, 118
Gentrichum sp, 203
Geranial, 75, 179
Geranyl diphosphate (GPP), 135
Geranyl diphosphate synthase (GPPS), 147, 164
Geranyl geranyl diphosphate (GGPP), 135
Geranyl pyrophosphate, 176
8-Geranyloxypsoralen, 95
Germacranolides, 44, 49, 51, 53, 306
Gibberellic acid (GA), 153
Gibbs reagent, 254
Giberella zeae, 209
Gingerol, 92, 320
Ginsenoside (Rg_1), 142
Ginsenoside production, 161
Ginsenoside Rh, 2, 159
Ginsenosides, 144, 158
Girdling, 165
Glabretal-type, 312
Glandular trichomes, 148
Glaucarubolone, 84
Glechoma hederacea, 11
Gliocadium roseum, 321
Globadera pallida, 99
Globodera rostochiensis, 72, 77, 81
Globodera rostochiensis, 77
Globularia alypum, 11
Gloriosa superba, 100
Glucides, 243
Gluconapin, 102
3'-*O*-β-D-Glucopyranosyl derivative, 194
5-*O*-α-D-Glucopyranosyl-5-hydroxymellein, 206
(3*S*)-8-(*β*-D-Glucopyranosyloxy)-3-[(1*E*,3*E*,5*E*)-hepta-1,3,5-trienyl]-3,4-dihydro-6-hydroxy-5-methyl-1*H*-2-benzopyran-1-one, 204
8-*O*-*β*-D-Glucopyranosylscorzocreticin, 197
Glucoraphasatin, 101
Glucosinolate sinigrin, 100
Glucosinolates, 100, 102
Glucotropaeolin, 101
Glutathione, 263
Glutathione (GSH), 123
Glutathione disulphide, 267
Glutathione peroxidase, 263, 267
Glyceollin, 94
Glyceraldehyde-3-phosphate (GAP), 177
Sn-Glycerol-1-eicosa-9,12- dienoate-2-palmitoleate-3- linoleate, 68
Glycine max, 11, 31, 94
Glycossomal glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 177
Golden rice, 165
Goniothalamim, 296
Goniothalamus andersonii Sinclair, 296
Gonoplebium incanum, 11
Gossypium hirsutum, 77
Gossypol, 77, 78
Grandisin, 292
Granuloma test, 57
Gratiola officinalis, 11
Ground state sensitizer, 261
Growth regulation, 238
Growth-inhibitor insecticides, 282

Growth-regulating properties, 278
GSSG, 267
Guaiacum officinale, 11, 28
Guaianolides, 44, 49, 50, 51, 53
Guaianolide-type sesquiterpene lactones, 306
Guineesine, 315, 366
Guttiferae, 201
Gynaecopathy, 47

H

H9c2 cardiac muscle cells, 125
Haber-Weiss reaction, 265
Haematic fits, 46
Haematuria, 45
Haeme–containing enzymes, 266
Haemonchus contortus, 55
Haemorrhage, 47
Haemorrhagic yellow fever, 238
Haemostatic agent, 48, 196
Hagenia abyssinica, 183
Haliclona cribricutis, 338
Haloxylon scoparium Pomel, 201
Hannoa undulata, 84
Hanphyllin, 53, 54
Headaches, 48
Hederagenin, 81
Hedycaryol, 307
HeLa (human epithelial carcinoma), 122
Helenium sp, 70
Helianthus annuus, 11
Helichrysum arenarium, 11
Heliotrine, 99
Helminthosporum maydis, 209
Hemidesmus indicus, 11
Hemigossypol, 77
Hemiterpene, 176
Hennosides, 243
Hepatic colic, 47
Hepatitis, 48
Hepatitis B, 139, 149
Hepatitis B and C virus, 55
Hepatoprotective, 48, 309
Heraclenin, 95
Herbal dietary supplements, 246
Herbicidal activities, 84, 207
Herbivorous spider mites, 137
Herniaria glabra, 11, 28
Herniaria hirsuta, 11
Herniarine, 295
Herpes, 48
Herpes viridae, 55
Heteradera schachtii, 101
Heterodera cajani, 75
Heterodera glycines, 69, 74, 94, 99
Heterodera schachtii, 101
Heterodermia leucomelos, 337
Hexadecanoylpyrrolidine, 315
β-Hexosaminidase, 211
Hibiscus sabdarifa, 11, 28
HIF-1a activity, 122
HIF-1a expression, 122
Histamine release, 211
HIV, 149
HIV-1 protease inhibitor, 309
HL-60 RG (human promyelocytic leukemia), 122
HMG-CoA reductase (HMGR), 152
Hongkongenin, 197, 198
Honokiol, 88
Horminone, 137
Hortonia angustifolia, 320
HPTLC, 220, 237, 252
  of Arctostaphylos products, 252
  of *Equisetum* spp, 237
HPTLC analysis, 231, 232
  of *Cassia senna* extracts, 231
  of commercial neem cake products, 232
  of Cynara scolymus, 231
  of neem (*Azadirachta indica*) products, 232
HPTLC fingerprint, 233, 241, 256
  in Chinese Pharmacopoeia, 256
  of Neem products, 241
Hsamaderines B, 84
HT-29 human colon cancer cells, 117
Human acute lymphoblastic leukaemia Molt-4, 62
Human African trypanosomiasis, 173
Human breast adenocarcinoma cell lines MCF7 and MKN, 59
Human cervical cancer, 119
Human cytomegalovirus, 55
Human hepatoma cells HepG, 2, 118
Human HL-60 cells, 120
Human lung adenocarcinoma ASTC-a-1 cells, 60
Human melanoma cell lines A375M and A375P, 59
Human melanoma MDA-MB-435, 62
Human promyelocytic leukemia, 62
Human prostate cancer 22Rv1cells, 127
Human prostate cancer LNCaP cells, 119
Human T-cell leukemia (MOLT-4), 122
Human umbilical vein endothelial cells (HUVEC), 125
α-Humulene, 77
Humulene epoxide II, 306

Humulus lupulus, 11, 28, 31
Huplothrips leucanthemi, 212
Hydrangea arborescens, 3, 11, 22
Hydrangea hortensia Smith, 191
Hydrangea macrophylla, 191
Hydrangea macrophylla, 209
Seringe var. *thunbergii* Makino, 209
Hydrangea species, 193, 211
Hydrangenol, 191
3*S*-Hydrangenol 4'-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside, 197
Hydrogen donors, 124
Hydrogen peroxide, 263, 264
Hydrogen-donating antioxidants, 262
Hydroquinone, 86, 252, 256
(2*R*,3*R*,4*R*,5*R*)-2,5-Bis (Hydroxy-methyl) pyrrolidine-3,4-diol, 98
2β-Hydroxy oleanolic acid, 81
6-Hydroxy plumbagin, 286
2-Hydroxy-1,4-naphtoquinone, 242
7-Hydroxy-2´,4´,5´-trimethoxy isoflavone, 303
3,4,6-(4'-Hydroxy-2'-methylphenoxy)-(3*R*)-mellein, 202
1-Hydroxy-2-methylanthraquinone, 291
(2R)-2-Hydroxy-2-phenylethylglucosinolate, 102
(3*R*)-8-Hydroxy-6-methoxy-3-undecyl-3,4-Dihydroisocoumarin, 196
4-Hydroxy-8-*O*-methylmellein, 207
4-Hydroxybenzoic acid, 86
4-Hydroxybenzylglucosinolate, 102
(2*S*)-2-Hydroxybut-3-enylglucosinolate, 102
Hydroxycoumarin derivatives, 295
Hydroxycyclohexene epoxides, 207
8α-Hydroxyelemol, 77
12-(2'-Hydroxyethyl) deoxoartemisinin, 59
(-)-3*β*-Hydroxylabdan-8(17)-en-15- oic acid, 308
Hydroxylase (L3H), 146
p-Hydroxymandelonitrile, 105
(+)-6-Hydroxymellein, 193
Cis-(3*R*,4*R*)-4-Hydroxymellein, 203
Hydroxymellein, 207
(3*R*)-7-Hydroxymellein, 207
5-Hydroxymellein, 207
5-Hydroxymethylfurfural, 73
2-(4-Hydroxyphenyl)-8-(3-methyl- but-2-enyl)- chroman-4-one, 93
6-Hydroxyramulosin, 193
6α-Hydroxyvouacapane-7*β*,7α- lactone, 309
Hygrophila auriculata, 11
Hygrophila spinosa, 11
Hygrophorus poetarum, 326
Hyperforin, 234
Hyperglucemia, 47
Hypericin, 234
Hypericum annulatum Moris, 201
Hypericum perforatum, 11, 28, 33, 235
Hyperoside, 235, 237
Hypertension, 25, 46, 142, 143
Hyphenated HPTLC, 257
Hypochaeris scarzonerae, 11
Hypocreaceae, 205
Hypocretenolides, 49
Hypoglycaemic, 48
Hypoxia, 153
Hyptis martiusii Benth, 303
Hystidine, 261

I

IcyHot, 146
IFN-γ, 129
IKKb phosphorylation, 129
IL-1β, 56
IL-6, 56
IL-8, 56
IL-8 release, 125
Ilex paraguariensis, 11
Ilex paraguensis, 314
Illuminated chloroplasts, 270
Immunodeficiency, 173
Immunosuppressive, 321
Imperatorin, 95
2D INADEQUATE analysis, 322
Indigofera tinctoria, 243, 244
Indole alkaloids, 96
Inducible enzymes, 128
Inducible nitric oxide synthase (iNOS), 54
Inflammation, 47, 48
Inflammatory cytokines, 56
Inflammatory enzymes, 128
Inflammatory mediators, 128
Inhibition, 59
of angiogenesis, 59
Inophyllin B, 285
iNOS, 56
iNOS expression, 56
Insecticidal, 84, 192, 279
Insecticidal amides, 316
Insecticidal bioassay-guided fractionation, 315
Insecticidal molecules, 338
Insecticide, 239, 279
α-V-β-3-Integrin expression, 59
Interferon-γ, 55
Interleukin-2 (IL-2), 55
Intermedeol, 307

Interneurons, 100
Intersystem crossing, 260
Intracellular Ca^{2+} level, 123
Intracellular free radicals, 262
Intracellular signaling molecule, 264
Inula helenium, 11, 23, 77, 307
Ion channels, 144
Ipomoea batatas, 163
Ipomoea turpethum, 11
Ircinia ramosa, 208
Irciniastatin A,B, 208
Iresina calea, 12
Iris germanica, 12
Iris japonica, 68
Iris versicolor, 12
Irradiation therapy, 144
Isoalantolactone, 307
Isobutylamide, 315
N-Isobutylamine alkaloids, 315
Isochromen, 191
Isoflavones, 269
Isolapachol acetate, 287
Isolapachol lithium salt, 287
(+/-)-Isomenthol, 144
Isoniazid, 210
Isonimocinolide, 309
Isopentenyl diphosphate (IPP), 176
Isopentyl diphosphate (IPP), 135
(-)-*trans*-Isopiperitenol, 146
(-)-Isopiperitenone, 146
(-)-Isopiperitenone reductase (IPR), 146
Isoplumbagolone, 286
Isoprenoids, 135
13-Isopropyl podocarpa-8,11,13- trien-12-ol, 79
4-Isopropylbenzyl alcohol, 303
2-Isopropyl-*para*-benzoquinone, 290
(+)-*cis*-Isopulegone, 146
Cis-Isopulegone isomerase, 146
Isoquercetin, 235
Isoquinoline alkaloids, 97
Isoshinanolone, 286, 287
Isothiocyanates, 100, 101

J

Jacaranda procera, 12
Japanese Yew, 155
Jatropha curcas, 12
Jatropha urens, 12
Jaundice, 46, 47, 48
Jinhenkang, 246
Juniperus communis, 12
Juniperus procera, 79

K

Kaempferol, 270
Kahweol, 141
Kanamycin, 210
Kazal motifs, 118
KB (human epidermoid carcinoma), 122
Keto reductases, 146
Kigelia pinnata DC, 193
Kigelin, 193
Kinase (ERK) activation, 125
Kinase pathways, 127
Klaineanone, 84
Knema hookeriana, 73, 106
Kojic acid, 326
Komaroviquinone, 175
Korean Ginseng, 158
Korean traditional medicine, 51

L

L3H enzyme, 147
L3H gene, 147
Labda-8(17),12-diene-15,16 dial, 308
Ent-Labdane diterpenoids, 308
Labiatae, 303
Lactones, 96
Lamiaceae, 125, 144
Lamium album, 12, 28
Lantacin, 80
Lantana camara, 80, 93
Lantanolic acid, 80
Lantanoside, 93
Lantoic acid, 80
Lapachol, 287
Laportea, 12
Larix decidua, 12
Larval habitats, 278
Larval mosquito susceptibility test, 364
Larvicidal, 279
Larvicidal activities, 279, 286, 298, 305
Larvicidal compounds, 282
Larvicidal coumarins, 295
Larvicidal toxicity, 309
Larvicide potency, 305
Laryngitis, 47
Lasiocarpine, 99
Laundry detergents, 144
Laurus azorica (Seub.), 207
Laurus nobilis, 12, 75
Lavendula anguistifolia, 12, 28
Lawsone, 242
Lawsonia inermis, 242
Lawsonia inermis L, 196
LDL oxidation, 124

LDL receptors, 120
Leakage of electrons, 263
Leguminosae, 297, 298, 300, 308
Leishmania amazonensis, 54
Leishmania braziliensis, 54
Leishmania donovani, 54
Leishmania infantum, 54
Leishmania mexicana, 54
Leishmania tropica, 54
Leonotis nepetaefolia, 12, 28
Leonotis ocymifolia, 183
Lepidium bipinnatifidum, 12
Lepidium sativum, 12
Lepidium virginicum, 12
Lepidoptera, 333
Lepoxygenase, 263
Leptadenia hastada, 12
Leptostachyol acetate, 292
Lespedeza capitata, 12
Leucopenia, 279
Leukemia, 155
Leukotrienes, 129
Levisticum officinale, 12
Lichens, 279
Lignan A, 272
Lignans, 88, 292
Lignification, 86
(*Z*)-Ligustilide, 320
Limonene, 75
d-Limonene, 119
(+)-*R*-and(-)-*S*-Limonene, 178
Limonene-10-al, 179, 185
Limonene-3-hydroxylase (L3H), 147
Limonin, 311
Limonoids, 313
Limonoids harrisonin, 311
Linalool, 75, 180
Linamarin, 105
Linaria vulgaris, 12
Linaroside, 93
Linear monoterpenes, 179
Linoleic acid, 272
Linolenic acid, 269, 270
Lipid membranes, 263
Lipid peroxidation, 124, 127, 261, 266
Lipid peroxidation by-products, 123
Lipid peroxides, 259
Lipooxygenase pathway, 128
Lipophilic character, 49
Lipophilic organic compounds, 127
Lipophilicity, 290
Lipoxygenase, 261
Lithraea molleoides, 92
Liver cancers, 119
Liver PON1 activities, 128
Lobelia chinensis, 12
Lomicera caprifolium, 12
Lonchocarpus, 98
Lonchocarpus chirinanus, 297
Longimicins A-D, 282
Longistylines C-D, 297
Lotaustralin, 105
Lotus corniculatus, 87
Lotus pedunculatus 87
Low caloric sweetener, 211
Low density lipoproteins, 120
LPS-induced COX-2, 56
LTB4 levels, 129
Luffa operculata, 12
Lung adenocarcinoma, 60
Lupinifolin, 298
Lupinifolin 4´-methyl ether, 298
Lupinus albus, 12
Lupinus angustifolius, 12
Lupinus mutabilis, 12
Lutein, 273
Luteolin, 129, 235, 271
Lycium halimifolium, 12
Lycopene, 137, 143, 273
Lycopersicon esculentum, 13, 28, 30
Lycopodium clavatum, 13
Lymph node carcinoma, 59
Lymphadenopathy, 279

M

Macleaya cordata, 97
Macleaya microcarpa, 98
Macrolide, 197
Macrophyllosides A, 194
Magnolia grandiflora, 78
Magnoliaceae, 49
Magnolol, 88
Magonia pubescens, 299
Mahanimbine, 314
Mahanine, 314
Malaria, 52, 139, 238
Malathion, 278
Malignant neoplasm, 58
Malonyl-CoA, 283
Malus baccata, 88
Malus domestica, 13, 28, 30
Mammary cancers, 119
Mammary carcinomas, 119
Mangifera indica, 163
Mangosharin, 286
α-Mangostin, 286

β-Mangostin, 286
Mangrove endophytic fungus, 207
Manilkara zapota, 13
Mansonia gagei, 320
Mansonia gagei Drumm, 289
Mansonone, 289
Mansonone C, 320
Margirocarpus setosus, 13
Maritinone, 286
Marmesin, 294
Marrubium vulgare, 13
Mass spectral libraries, 304
Matrine, 97
Medicagenic acid, 81
Medicago sativa, 81, 94
Medicago species, 81
Medicarpin, 94
Mefloquine, 149
Melaleuca alternifolia, 182, 187
Melanoma cells, 117
Melia azedarach, 13, 68, 73
Meliaceae, 320
Melicope subunifoliolata, 298
Melilotus dentatus Waldst e Kant, 206
Melilotus officinalis, 13
Melissa officinalis, 182
Meliternatin, 299
Mellamide, 325
Mellein, 212
Meloidogyne arenaria, 74
Meloidogyne hapla, 99, 101, 105
Meloidogyne incognita, 68, 69, 70, 73, 80
Meloidogyne javanica, 75, 84, 88, 93
Meloidogyne spp, 101
Membrane disruption, 261
Membrane-associated enzyme, 264
Menstrual pain, 45
Menstruation, 47
Menstruation-related problems, 46
Mentha aquatica, 144
Mentha arvensis, 148
Mentha piperita, 144, 146, 147
Mentha spicata, 13, 28, 144, 187
p-Menthans, 182
Menthofuran, 147
Menthofuran synthase gene, 147
Menthol, 75, 144
(+)-Menthol, 144
Mentholatum, 146
(-)-Menthone reductase (MR), 146
Mentzelia hispida, 13
MEP (methylerythritol-4-phosphate), 135
Mercurialis annua, 13
Mercurialis perennis, 13
Meroterpenoids, 289
Metabolic engineering, 147
Metalloproteinase-2 production, 59
Metalloproteinases, 118
Metam sodium, 101
Metamorphosis, 281, 289
Metarhizium anisopliae, 278
Metastasis, 58, 60, 117
Methionine, 261
Methoprene, 278
7-Methoxy coumarin, 295
3-Methoxy-4-hydroxy benzoic acid, 87
(*S*)-4-Methoxy-7-phenyl-7,8-dihydro[1,3] dioxolo[4,5-*g*]isochromen-5-one, 201
6-Methoxygossypol, 77
6-Methoxyhemigossypol, 77
6-Methoxymellein, 192, 193
4-(2-Methoxyphenyl) piperazine, 119
1-(3´-Methoxypropanoyl)-2,4,5-trimethoxybenzene, 293
3-Methoxytanapartholide, 50, 57
Methyl 4-hydroxycinnamate, 86
Methyl 6α,*β*-dihydroxyvaoucapan-17*β*- oate, 309
Methyl benzoate, 87
O-Methyl carvacrol, 180
6-*O*-Methylscorzocreticoside, 197
3-*O*-Methyl droserone, 286
O-Methyl espintanol, 180
Methyl ferulate, 91
Methyl isothiocyanate, 101
Methyl jasmonate, 158, 161
Methyl propyl trisulfide, 106
Methyl viologen, 270
5-Methyl-2,2´,5´,2´´-terthiophene, 284
Methyl-4-(1-methylethyl)- (1*R*,3*R*,4*S*)-1-cyclohexan-3-ol, 144
3,4-*trans*-3-Methyl-4,6,8-trihydroxy-dihydroisocoumarin, 203
3*R*-Methyl-5,6-dimethoxy-7,8-methylenedioxy dihydroisocoumarin, 193
N-Methyl-6*β*-methoxy-2*β*-methylpiperidine, 314
3-Methyl-6,8-dimethoxy-3,4-dihydroisocoumarin, 205
3-Methyl-6-methoxy-3,4-dihydroisocoumarin-8-*O*-*β*-D-glucopyranoside, 212
6-Methyl-6-methoxy-3,4-dihydroisocoumarin-8-*O*-*β*-D-glucopyranoside, 200
3*R*-Methyl-6-methoxy-7,8-methylenedioxydihydro-isocoumarin, 193

3-Methyl-8-methoxy-3,4-dihydroisocoumarin, 205
6-*O*-Methylannulatomarin, 201
2-Methyl-benzoquinone, 290
N-Methylcytisine, 97
Methylenedioxyundecatrienoylpiperidine, 316
Methyleugenol, 293
3-O-Methyl-iso-secotanapartholide, 50
N-Methyl-*N'*-nitro-*N*-nitrosoguanidine, 118
23-*O*-Methylnimocinolide, 309
2-Methyl-*para*-benzoquinone, 290
Methylpiperidine, 314
4-Methylsulfinylbut-3-enylglucosinolate, 102
Mevalonate, 176
Mevalonate depletion, 176
MIA PaCa-2 cell proliferation, 126
Miami *botanicas*, 3
Micellar solubilization, 127
Michael acceptors, 57
Michael-type acceptor, 55
Microaerophilic bacterium, 266
Microarray analysis, 60
Microbicidal, 209
Microbotryum violaceum, 206, 210
Microcos paniculata, 314
Microcystis aeruginosa, 336
Microplate Alamar Blue Assay (MABA), 210
Microsphaeropsis sp, 207
Microsporum canis, 210
Microtubule assembly, 155
Millettia dura Dunn, 300
Millettone, 300
Millettosin, 300
Mint odour, 144
Mitochondrial apoptotic pathway, 122
Mitochondrial dysfunction, 123
Mitochondrial lipids, 272
Mitochondrial morphology changes, 60
Mitochondrial thioredoxin system, 267
Mitocondrial membrane potential, 118
Molinema dessetae, 73
Momordica charantia, 13, 28, 33
Monarda didyma, 13, 23
Mongolian folk medicine, 47
Monimiaceae, 320
Monocrotaline, 99
Monocytes/macrophages, 56
Monooxygenase, 269
Monoterpene hydroperoxides, 177, 181, 186
Monoterpenes, 116, 176
Monoterpenoids, 305
Montroumarin, 197
Montrouziera sphaeroidea Pancher Ex Planchon et Triana, 197
Morin, 270
Morinda citrifolia, 291
Morinda geminata, 13, 28
Morinda longiflora, 13, 28
Morinda lucida, 13, 28
Morindone-6-methyl-ether, 291
Moringa stenopetala, 183
Moronic acid, 142
Mosquito *Aedes aegypti*, 277
Mosquito larvicidal butenolides, 320
Mosquito repellent, 48
Mosquitocidal, 279, 314
Mosquitocide, 279
Mosquito-repelling activities, 303
Motor neurons, 100
Mountain wormwood, 47
Mouse osteoblasts, 128
Moxartenolide, 57
mRNA expression, 117
Mucuna aterrima, 69
Mucuna pruriens, 13, 28, 33
Mullein, 205
Multi-drug resistance, 159
Multiwavelength chromatogram, 224
Multiwavelength densitometry, 222
Murine epithelial intestinal cells, 129
Murisolin, 73
Murraya koenigii, 314
Murrayanol, 314
Mustard plants, 100
Mutation induction, 146
MVA (mevalonate), 135
MVA pathway, 135, 152
Myalgia, 279
Mycobacterium tuberculosis, 210
Mycotoxins, 207
Mycotypha microspora, 209
Mycrocentrospora acerina, 212
Mycrocystin-RR, 336
Myeloperoxidase, 264
Myricetin, 270
Myristicin, 293
Myroxylon balsamum, 293, 306
Myrrhis odorata, 13
Myrsinaceae, 289

N

NADH:ubiquinone oxidoreductase, 303
NADPH, 266
NADPH dependent limonene-3-hydroxylase (L3H), 146

NADPH dependent P-450-iron-containing enzyme, 301
1,4-Naphthoquinone, 287
Naphthoquinone derivatives, 287
Naphthoquinones, 289
Narcissus pseudonarcissus, 165
Naringin, 298, 366
Naringoside, 298
Nasturtium officinale, 13
Natural antioxidants (NAO), 122, 259, 267, 274
Natural insecticides, 282
Natural polyacetylenes, 283
Nauclea latifolia, 13, 28
Necator infections, 100
Neem cakes, 239
Neem oil, 239
Neem (*Azadirachta indica*), 232
Nematicidal dialkyl succinates, 68
Nematicidal prenylated flavanones, 93
Nematicidal thiophenes, 72
Nematode management, 108
Nematode mortality, 91
Neoaustin, 325
(+/-)-Neoisomenthol, 144
Neolignans, 292
(+/-)-Neomenthol, 144
Neonatal hyper bilirubinemia, 243
Neoprocurcumenol, 320
Neovascularization, 122
Nepeta cataria, 179
Nephrotoxic, 321
Neral, 179, 185
Nerium indicum, 85
Nerium oleander, 13, 28, 33
Nerol, 179
E-Nerolidol, 306
Neuralgia, 46
Neurodegenerative diseases, 124, 259
Neuroectodermal tumour cells, 117
Neurolaena lobata, 13
Neurolenin B, 175
Neurons, 56
Neutrophils, 264
NF-jB activity, 123, 126–127
NF-jB activity suppression, 127
NF-kappa B, 128
NF-κB activation, 128, 129
NF-κB inhibition, 57
NF-κB p, 65, 129
NF-κB transcriptional activities, 57
NF-κB/p65 transcriptional activity, 127
Nicotine, 99
Nifurtimox, 174, 178
Nifurtimox-eflornithine, 174
Nigella sativa, 13, 28
Nimbin, 239
Nimbocinol, 311
Nimesulide, 248
Nimocinol, 309
Nimocinolide, 309
Nimolicinol, 311
Nitric oxide, 56, 263, 265
Nitric oxide synthases, 263
Nitroaryl-substitute polyketides, 327
Nitrogen oxygen species (RNOS), 259
N-nitrosodiethylamine, 120
Nodulisporic acid, 322, 323
Nodulisporium sp, 205, 206, 322
Non-photochemical reaction, 261
Non-phagocytic cells, 264
Non-propagating radical, 262
Non-ribosomal peptides, 279
Non-sporulating fungus, 322
Nordamnacanthal, 291
(-)-Nortrachelogenin, 91
Nortrachelogenin triacetate, 91
Nor-triterpenes, 239
Notopterygium forbesii Boiss, 198
Novel antioxidants, 274
N-ras oncogene, 119
Nuclear receptor responsiveness, 59
Nuclear transcription factor activation, 128
Nuclear transcription factor kappaB (NF-κB), 56
Nutraceuticals, 129
Nymphaea lotus, 13

O

Ochratoxin A, 321, 326
Ochratoxins, 193, 203
Ocimum basilicum, 13, 31, 183
Ocimum gratissimum, 81
[(2*E*)-Octadecanoyl]-pyrrolidine, 315
Odoratrin, 79
Olea europaea, 13, 28, 88
Oleanolic acid, 81, 175, 309
Ononis spinosa, 13
Opilia celtidifolia, 13
Oral cancer cell line YD-10B, 59
Orcinol, 86
Organic free radicals, 261
Origanum vulgare, 14, 28
Orthosiphon aristatus, 14
Orthosiphon spicatus, 14, 28
Orthosiphon stamineus, 14

Oryza sativa, 165
Oscillatoria agardhii, 337
Ostertagia circumcincta, 100
Otivarin, 73
Ovarian cancer, 155
Overexpression, of phytoene synthase, 165
Ovicidal activity, 238, 298
Oviposition deterrent, 298
Oxandra espintana, 180
Oxidative damage, 260, 261
Oxidative damage in plants, 261
Oxidative phosphorylation, 303
Oxidative stress, 259
Oxidisable substrate, 262
Oxidized low-density lipoprotein (oxLDL), 124
Oxidosqualene, 160
Oxoaporphine alkaloid liriodenine, 314
Oxygen radicals, 120
Oxygenated guaianolides, 62
Oxypeucedanin, 294
Oxypeucedanin hydrate, 294
Oxytocic, 256
Oxyuriasis, 77

P

P-388 D1 (mouse lymphoid neoplasma), 122
p38MAPK, 123
P450 monooxygenase, 150
p53 activation, 127
P65 phosphorylation, 129
Paclitaxel, 59, 155
Panagrellus redivirus, 72, 78
Panagrellus redivivus, 85, 88, 91
Panagrolaimus sp, 97
Panax ginseng, 158
Panax quinquefolius, 14, 28, 31, 158
Panc-1 cell proliferation, 126
Pancreatic cancer, 126
Pancreatic cancer cell proliferation, 126
para-Cymene derivatives, 180
Para-benzoquinone, 290
Paraoxonase (1 PON1) expression, 128
Paraphenylendiamine, 243
Paraquat, 270
Para-quinone, 252
Parasite mitochondrian, 53
Parasite-encoded sarcoplasmic-endoplasmic reticulum calcium ATPase, 53
Parasitic glycolysis, 175
Paratrichodorus minor, 74
Parietaria judaica, 14
Parietaria officinalis, 14
Parkinson's disease, 259
Parmotrema pseudotinctorum, 338
PARP, 118
PARP cleavage, 118
Parquetina nigrescens, 14
Partition coefficients, 262
Passiflora jorullensis, 14
Pastinaca sativa, 14
Pauli Exclusion Principle, 260
Paullinia cupana, 14, 314
PDE-5 inhibitors, 247
Pectoral, 48
Pedonin, 311
*Pelargonium graveolen*s, 75
Pellitorine, 315, 366
Penicillium citrinum, 326
Penicillium sp, 208
Penicillum expansum, 209
Pentadecane, 320
Pentahydroxy aglycones, 270
Pentahydroxydihydrochalcone, 272
Pentamidine isethionate, 175
(−)-(*R*)-(3)-*trans*-Pentenyl-(3)-5-*n*-butyl-6,8-dihydroxy-3,4-dihydroisocoumarin, 203
Peppermint, 147
Peptic ulcer, 46
Peptides, 336
Permethrin, 278
Peroxiredoxins, 263, 264, 267
Peroxocarboxylate, 265
Peroxofabiane, 152
Peroxonitrous acid, 265
Peroxy radicals, 261
Peroxynitrite, 124, 265
Persea americana, 14
Pestalotiopsis microspora, 157
Pesticides, 144
Petasites hybridus, 14
Petroselinum crispum, 14, 25, 28
Peucedanum ostruthium, 14
Peumus boldus, 14, 28
Phagocytic cells, 263
Phagosomes, 264
Phalanis canariensis, 271
Pharyngitis, 47
Phaseolus lunatus, 94, 137
Phaseolus vulgaris, 14, 28
Phasmarhabditis hermaphrodita, 99
2-Phenethylglucosinolate, 102
Phenol derivatives, 293
Phenol α-tocopherol (Vitamin E), 261
Phenolic acids, 121, 271

Phenolic antioxidant, 124, 268
Phenolic benzochroman derivatives, 268
Phenyl propanoids, 185
2-Phenyl-5-(1'-propynyl)thiophene, 70
Phenylalanine ammonia lyase, 86
2-Phenylethyl glucosinolate, 102
2-Phenylethyl isothiocyanate, 101, 102
1-Phenylhepta-1,3,5-triyne, 70
Phenylpropanoid derivatives, 91
Phenylpropanoids, 88, 279, 293
Phenylpropenes, 293
3-Phenyl-substituted DHIC, 201
Phleum pretense, 68
Phloroglucinol, 86
Phomolactones A, 207
Phomopsis, 207
Phomopsis helianthi Munt-Cvetk, 203
Phomosines, 207
Phompopsin A, 207
Phosphorylation, 56, 127
Photobleaching, 270
Photochemical reaction, 260
Photodynamic, 260
Photoexcited compounds, 260
Photo-oxidative damage, 163, 260
Photoperoxidation, 271
Photosensitizers, 273
Photosynthesis, 153
Photosensitizers compounds, 283
Phototoxic effect, 366
Phryma leptostachya var. *asiatica*, 292
Phyllanthus amarus, 14
Phyllanthus niruri, 93
Phyllanthus salviaefolius, 14
Phyllodulcin, 191, 193, 209, 211
(3*R*)-Phyllodulcin, 193
3*S*-Phyllodulcin 8-*O*-b-glucoside, 194
Physalis alkekengi, 14
Physalis peruviana, 14
Physical quenchers, 261
Physostigma venenosum, 96
Physostigmine, 96
Phytoalexin production, 94
Phytoalexins, 94
Phytoene desaturase, 164
Phytoene synthase, 165
Phytoene synthase gene, 165
Phytophtora infestans, 206
Phytoseiulus persimilis, 137
Phytovigilance, 246
PI3K, 123
PI3-kinase-dependent Pin1 expression, 126
Picria fel-terrae, 14
Pigmentation, 137
Pimenta dioica, 183
Pimpinella anisum, 14, 29, 75
Pimpinellin, 294
α-Pinene, 75
β-Pinene, 75
Pinenes, 182
α-and*β*-Pinenes, 305
(+)-Pinoresinol, 91
Pinosylvin monomethyl ether, 91
Pinus massoniana, 77, 91
Pinus palustris, 91
*Pinus strobe*s, 91
Pinus sylvestris, 14
Pipbinine, 316
Piper aduncum L, 208
Piper amides, 317
Piper betle, 88
Piper cubeba, 14
Piper fimbriulatum, 292
Piper guineense, 14, 29
Piper longum, 14
Piper methysticum, 15, 29, 33
Piper nigrum, 315
Piper solmsianum, 293
Piper umbellatum, 15
Piperaceae, 292, 315
Pipercide, 366
Pipercitine, 315
Piperidine alkaloid, 314
Piperine, 315
Piperitone, 187
Pipernonaline, 315
Piperonal, 293
Pipertipine, 315
Pipgulzarine, 315
Pipnoohine, 315
Pipsaeedine, 316
Pipwaqarine, 316
Pipyahyine, 315
Pipzorine, 315
Piquera trinervia Cav, 183
Piquerol A, 183
Pistacia terebinthus, 75
PKCd, 127
Planar chromatography, 223
Plant growth inhibition, 192
Plant-parasitic nematodes, 67
Plasmodium falciparum, 44, 52, 74, 149, 203, 210
Plasmodium malariae 52
Plasmodium ovale, 52
Plasmodium vivax, 52

Plastid, 135
Plastidial MEP pathway, 156
Platelet aggregation, 127
PLC/PRF/5 (human hepatoma), 122
Pleiotropic response, 59
Plocamium cartilagineum, 177
Plumbagin, 286, 290
Plumbago capensis, 286
Plumeria rubra, 15
Poecilia reticulata, 340
Polyacetylene derivative, 366
Polyacetylenes, 70
Polygala hongkongensis Hemsl, 198, 210
Polygonaceae, 298, 299
Polygonium senegalensis, 298, 299
Polygonum acre, 15
Polygonum hydropiper, 15
Polyhydroxylated chalcones, 272
Polyketide assembly, 336
Polyketide pathway, 290
Polyketides, 321, 366
Polymerized tubulin, 155
Polyp numbers, 127
Polyphenol oxidases, 124
Polysulfonated naphthylamine, 175
Pometia pinnata, 15
Pomolic acid, 80
Poncirin, 298, 366
Poncirus trifoliata, 294, 298
Populus nigra, 15
Populus tremuloides, 15, 29
Porrifolius, 197
Portulaca oleracea, 15, 29
Post-transcriptional actions, 120
Potato cyst nematode, 72, 91
Potent inhibitors, 176
Pothomorphe peltata, 15
Pratylenchus coffeae, 64, 66
Pratylenchus neglectus, 102
Pratylenchus penetrans, 68, 70, 72, 94, 99, 101
Pratylenchus scribneri, 94
Pratylenchus scribneriv, 94
Preaustinoid A, 325
Preaustinoid A, 2, 325
Preaustinoid B, 325
Prenyl diphosphate, 135, 156
Prenyl transferase, 164
Prenylated stilbenes, 297
Primary antioxidant, 263
Primula veris, 15
Pro-apoptotic activity, 117
Pro-apoptotic proteins, 127
Probucol, ascorbic acid, 125
Procarbazine, 118
Pro-inflammatory cytokines, 128
Pro-inflammatory gene promoters, 128
Promoter-linked luciferase, 59
Promyelocytic leukemia cells (HL-60), 117
Propagation, 261
Propanyl-CoA, 283
Prophylactic vaccine, 175
Prostaglandins (PGs), 56
Prostate LNCaP, 59
Prostatitis, 31
Protective immunization, 175
Protein isoprenylation, 120
Protoanemonin, 31
Protocatechuic acid, 122
Protopine, 98
Protozoa *Trypanosoma*, 173
Prunus avium, 15
Prunus cerasus, 15, 30
Prunus spinosa, 15
Psammaplysilla purpurea, 338
Pseudognaphaliu obtusifolium, 15
Pseudoguaianolides, 49
Pseudohypericin, 235
Pseudomonas fluorescens, 337
Pseudomonas picketti, 209
Psoralea corylifolia, 15
Psoriasis, 140, 141
Psymberin, 208
Pterodon polygalaeflorus (Benth), 309
Pulegone, 147
Pulmonaria officinalis, 15
Pulmonary adenoma formation, 120
Pulmonary diseases, 46
Purgative, 25
Purine alkaloid caffeine, 314
Purpureacin, 1, 2, 282
Purpurin, 291
Pycnanthus angolensis, 90
Pyrazolepyrimidine moiety, 248
Pyrenomycetes, 148
Pyretrins I, 278
Pyretrins II, 278
Pyricularia oryzae Cavara, 193
Pyriproxyfen, 278
Pyroangolensolide, 311
Pyrocatechol, 86
Pyrococcus furiosus, 266
Pyrogallol, 87
Pyrrolidine alkaloids, 98
Pyrus communis, 15, 31
Pyxine consocians, 337

Q

Qinghaosu, 52, 148
Quassia indica, 84
Quassinoids, 84
Quercetin, 93, 126, 235, 269, 299
Quercetin-loaded microemulsion, 129
Quercus calliprinos, 15
Quinine, 149
Quinizarin, 291
Quinoline-resistant parasites, 149
Quinolines, 149
Quinolizidine alkaloids, 97
Quinolone alkaloid, 100
Quinones, 137, 279

R

Rac, 1, 123
Radiation-induced apoptosis, 120
Radiation-induced apoptotic pathway, 120
Radical chain reaction, 261
Radical oxygen, 150
Radical scavenging activity, 274
Radioprotective agent, 144
Rafnia perfoliata, 15
Ranunculin, 31
Ras, 123
Ras oncoprotein plasma membrane, 120
Ras-activating medium, 119
Rash, 279
Reactive nitrogen species (RNS), 259
Reactive oxygen species (ROS), 122, 124, 261
Rearrangement, 313
Red1 transcription, 152
Redox potential, 262
Regulatory aspects, 219
Regulatory documents, 233
  on medicinal plants, 233
Rehmannia lutea, 15
Renal colic, 46
Repellence, 238, 278, 319
Reserpine, 250
Resistant alfalfa cultivars, 94
Respiratory electron transport chain, 303
Reticulol, 193
Retrofractamide A, 315, 316, 366
Retrofractamide D, 316
Retrosynthetic analysis, of artemisinin, 154
Retusin, 300
Reversion-inducing cysteine rich protein, 118
Rhabditis sp, 97, 99
Rhacoma uragoga, 15
Rhamnetin, 270
8-*O*-[a-L-Rhamnopyranosyl-(1→6)-b-D-glucopyranosyl]scorzocreticin, 197
Rheo spathacea, 29
Rheumatic diseases, 47
Rheumatic pain, 46
Rheumatism, 45, 47
Rheumatoid arthritis, 47
Rhinitis, 248
RhoA,B, 123
Rhododendron aureum, 15
Rhododendron campylocarpum, 15
Rhododendron ferrugineum, 15
Rhododendron ponticum, 16
Rhoeo spathacea, 15, 24
Rhoifolin, 298, 366
Rhycholacis penicillata, 91
Rhynchonin A,B, 90
Ribes nigrum, 16, 29, 31
Ricqlès, 146
Ridentin, 53, 54
Rishitin, 77
Robinetin, 270
Rollinia leptopetala, 314
Rolliniastatin, 282
ROS associated VEGF expression, 122, 125
ROS generation, 117
ROS molecules, 263
Rosa canina, 16
Rosmaridiphenol, 272
Rosmarinic acid (α-*O*-caffeoyl-3,4-dihydroxyphenyl lactic acid), 125
Rosmarinus officinalis, 16, 29, 30, 272
Rotenoid producing species, 300
Rotenone, 300, 303
Rotundial, 319
Rotylenchulus reniformis, 80, 86
Rotylenchulus reniformis, 93
Rubefacient, 25
Rubia tinctoria, 3
Rubia tinctorum, 16
Rubiaceae, 314, 320
Rubus niveus, 87
Rudbeckia hirta, 70
Rumex acetosa, 16
Rumex acetosella, 16
Rumex obtusifolius, 87
Ruscus aculeatus, 16
Rutaceae, 295, 298, 312, 314
Rutin, 235, 237
Ryanodane diterpene, 309

S

Sabinene, 182, 186
Saccharomyces cerevisiae, 154
Saccharopolyspora spinosa, 333
Safflower, 70
Safrole, 185, 187, 293
Salannin, 239
Salicylaldehyde, 293
Salicylic acid (SA), 153, 158
Salvadora oleoides Decne, 198
Salvadora persica, 16, 29
Salvadorin, 198
Sambucus canadensis, 16
Sambucus ebulus, 16, 29
Sambucus nigra, 16
Sambucus simpsonii, 16
Sanguinarine, 98
Santalum album, 16
(+)-Santonide, 55
Sapindaceae, 299, 314
Saponin pathways, 161
Saponins, 31, 80, 158
Sarmentine, 315
[(2*E*,4*E*)-Sarmentine, 315
Sarotherodon niloticus, 340
Sassafras albidum, 16, 29
Sassafras officinale, 187
Satureja macrantha, 180
Saxifraga montana H, 200
Saxifragaceae, 201
Scabies, 47
Scavengers, 259
  of free radicals, 259
Scavenging ability, 163
  of carotenoids, 163
Scavenging activities, 274
Schistosoma mansoni, 100, 150
Schistosomal infections, 150
Schistosomiasis, 139, 149
Scorzocretici, 197
Scorzocreticin, 197
Scorzocreticoside I, 197
Scorzocreticoside II, 197
Scorzonera, 197
(±)-Scorzotomentosin, 197
(-)-Scorzotomentosin 4'-*O*-β- glucoside, 197
Scrophularia nodosa, 16
Scutellaria lateriflora, 16, 29
Secale cereale, 68
Secoguaianolide, 51, 53
Second singlet states, 260
Secotanapartholide C, 50, 51
Seco-tanapartholides, 56
Sedanolide, 96
Sedatives, 45
Seed gall nematode (*Anguina tritici*), 72
Seed germination, 176
Selaginella lepidophylla, 16
Selaginella pilifera, 16
Selaginella pringlei, 16
Selenicereus grandiflorus, 16
Semi-synthetic artesunate, 150
Senecio aureus, 16
Senecio jacobaea, 99
Senecionine, 99
2-Senecioyl-4-vinylphenol, 297
Senkyunolide J, 96
Senkyunolide N, 96
Sensitizers, 260
Sensual tea, 246
Septoria tritici, 206
Serenoa repens, 16, 29, 33
Serenoa serrulata, 3
Serpentine, 96
Serum homocysteine thiolactonase (HCTL), 128
Sesanum indicum, 272
7-epi-Sesartemin, 293
Sesquicillium candelabrum F-114, 321
Sesquiterpene biosynthesis, 176
Sesquiterpene lactone, 49
Sesquiterpene lactone artemisolide, 62
Sesquiterpene lactone endoperoxide, 43
Sesquiterpene lactones, 44, 49
Sesquiterpenes, 175
Sesquiterpenoids, 77
[6]-Shogaol, 92
Short-lived singlet, 260
Signal transduction pathways, 126
Sildenafil, 247
Silymarin, 270
Simaroubaceae family, 84
Sinapic acid, 122
Sinapis alba, 16, 101
Sinapis spp, 100
Sinarundinaria nitida, 204
Singlet oxygen ($^1O_2$), 260
Singlet state, 260
Singlet oxygen quenchers, 124
Singlet oxygen scavenger, 152
Sinigrin, 100
Site-specific mutations, 59
Skin carcinogenesis, 118
Skin carcinogenesis mice model, 118
Skin diseases, 48
Sleeping sickness, 174

S-methyl propanethiosulfonate, 106
Smilax lanceaefolia, 274
Smilax officinalis, 16, 29
Smilax utilis, 16
Sodium anacardate, 293
Soil fumigant, 100
Solanum dulcamara, 16, 33
Solanum nigrum, 17, 33
Solanum seaforthianum, 17
Solanum surattense, 17
Solanum toryum, 17
Solavetivone, 77
Solenopsis invicta, 212
Solidago altissima, 70
Solidago canadensis, 17, 29
Solidago virgaurea, 17
Somaclones, 146
Sopanaria officinalis, 17
Sophocarpine, 97
Sophora alopecuroides, 97
Sophora flavescens, 97
Sophoramide, 97
Sorbaria sorbifolia, 88
Sore throats, 48
Sores, 47
Sorghum bicolor, 105
Sorghum bicolor, 104
Sorghum sudanense, 105
Soyasapogenol B, 81
Soybean roots, 94
Sp1 transcription factor, 59
Spasmolytic, 45, 48
Spathelia excels, 312
Spathulenol, 307
Spectinabilin, 327
Spice flavonoids, 125
Spinosad, 366
Spinosad suspension, 366
Spinosyn D, 336
Spinosyns A, 334
Spinosyns analogs, 331
Spiraea formosana Hayata, 196
Spiraea salicifolia, 88
Spiranthes diuretica, 17, 25, 29
Spodoptera eridania, 55
Sponges, 338
Sporormia affinis Sacc, 193, 202
Sporormia bipartis, 202
Squalene, 143
Squalene 2,3-oxide, 313
Squalene epoxidase, 160, 161
Squalene synthase (SS) gene, 161
St John's wort, (*Hypericum perforatum*), 233
Staphylococcus aureus, 55, 209
STAT3 activity, 122
STAT3- inducible VEGF gene expression, 122
Sterculia guttota, 320
Sterculiaceae, 314, 320
Steroidal glycoalkaloids, 85
Stomachic, 47, 48
Streptomyces avermitilis MA-4680, 329
Streptomyces globisporus, 336
Streptomyces thioluteus, 327
Strongyloides ratti, 98
Strophanthus kombe, 17
Styrene oxide, 185, 187
Styryldihydropyrone, 296
Styrylpyrones, 296
Sudorific, 25
Sulfated glycoprotein, 175
Sun protectants, 163
Superoxide, 263, 265
Superoxide dismutase (SOD), 263, 264, 266
Superoxide reductase, 263
Superoxide reductases (SOR), 266
Superoxide-promoted redox reactions, 270
Superoxides, 164
Suramin, 182
SW620, 62
Sweetener properties, 211
Swietenocoumarin B, 294
Sylvaticin, 282
Synergistic effect, 129
Synthesis, 153
  of artemisinin, 154
Synthetic pyrethroids, 282
Syringaresinol, 91
Syringic acid, 87, 122
Syzygium aromaticum, 183

T

Tabebuia heterophylla, 17, 29
Tadalafil, 247
Tagetes erecta, 17, 71, 284
Tagetes lucida, 17, 72, 295
Tagetes minuta, 72
Tagetes multifida, 17
TAM-resistant breast cancer cells, 126
Tamsulosin hydrochloride, 35
Tangeterin, 300
Taraxacum officinale, 3, 17, 25, 29
Targionaceae, 306
Targionia lorbeeriana, 306
Taverniera abyssinica, 94
Taxa-4(5),11(12)-diene (taxadiene), 156

Taxaceae, 155
Taxadiene production, 157
Taxadiene synthase, 156
Taxoids, 156
Taxol, 43, 141, 155
Taxol's anticancer activity, 156
Taxomyces andreanae, 157
Taxus brevifolia, 155
Taxus cell cultures, 158
Taxus chinensis, 155
Taxus cuspidata, 155
Tectoquinone, 287
Teladorsagia circumcincta, 87
Temephos, 278, 339
Tephrosia, 300
Tephrosia purpurea, 17, 29
Tephrosia toxicaria Pers, 300
Tephrosin, 300
Teratogenic, 321
Terazosin hydroc, 35
c-Jun N-Terminal (JNK) kinase, 125
Terminalia belerica, 17
Terminalia ivorensis, 17, 29
Terminalia laxiflora, 17
Terminalia macroptera, 17
Termination phase, 261
Terpenoids, 279, 303, 366
Terpinen-4-ol, 182, 187
γ-Terpinene, 120
α-Terpineol, 75, 176
α-Terpinolene, 75
Terpinolene, 182
α-Terthiophene, 72
Tetra-*cis*-lycopene, 164
Tetracyclic triterpene (dammarane) glycosides, 158
5,6,7,7a-Tetrahydro-3H-pyrrolizin-3-one, 336
5,6,7,7a-Tetrahydro-3H-pyrrolizin-7a-ol-3-one, 336
7,8,15,16-Tetrahydrodibenzo [c, i][1, 7] dioxacyclododecine-5, 13-dione ring system, 197
Tetrahydroepoxyazadiradione, 311
Tetrahydrogedunine, 311
Tetrahydroxyflavone, 126
Tetranor-triterpenoid limonoids, 239
Tetranortriterpenoids, 309
Tetranychus urticae, 137
Tetravalent chimeric dengue vaccine, 280
Thea sinensis, 314
Theaceae, 314
Theobroma cacao, 17, 29, 314
Theobroma grandifora, 211
Thespesia populnea, 17
Thiamine (vitamin B-1) deficiency, 31–32
Thiarubrines, 70
Thiazide diuretics, 3
Thioacetamide (TA)-induced hepatic injury, 118
Thioglucose conjugates, 101
β-Thioglucosidases, 101
Thiohydroximate-*O*-sulphonate, 101
Thiophene derivatives, 283
Thioredoxin peroxidases, 267
Thioredoxin reductase, 268
Thiosildenafil, 247
THP-1 mammalian cell lines, 181
Three dimensional visualisation, 237, 244
Thuja occidentalis, 17
β-Thujaplicin, 303
Thujone, 187
Thunbergii Makino, 191, 209
Thunberginol G 3'-*O*-β-glucoside, 194
3*R*-3*S*-Thunberginol I 8-*O*- glucosides, 194
Thunberginols C, 194
Thuringiensis var *israelensis*, 339
Thymol, 75, 180, 186, 305
Thymoquinol methyl ether, 180
Thymoquinone, 290
Thymus vulgaris, 17, 29, 31, 182
Tiger Balm, 146
Tilapia mossambica, 340
Tilia platyphyllos, 17
Tiliaceae, 314
TLC visualizer, 221
α-Tocopherol, 164, 268
α-Tocopherol protectant, 124
Tocopherols, 164
Toll-like receptors-NF-κB signalling pathway, 128
Tolypocladium tundrense, 326
α-Tomatine, 85
Tonsillitis, 48
Topoisomerase I and II, 314
Topoisomerase II inhibitor, 118
Total synthesis, 154
  of artemisinin, 154
Totarol, 79
Toxic singlet oxygen, 285
Toxicity, 292, 341, 360, 362
  of bacteria metabolites against *Ae. aegypti*, 362
  of fungal metabolites against *Ae. aegypti*, 360
  of metabolites produced by lichens against *Ae. aegypti*, 363
  of plant compounds against *Ae. aegypti*, 341
  to mosquito larvae, 292

Toxigenic mold fungi, 33
Tragopogon porrifolius L. subsp. *porrifolius*, 197
TRAIL receptors, 126
Tranquilizer, 48
Trans-3-methyl-4,6,8-trihydroxy-3,4-dihydroisocoumarin, 203
Trans-4,6-dihydroxymellein, 203
Trans-anethole, 75
Trans-cinnamic acid, 89
Transcription 3 (STAT3), 122
Transcriptional activation, 129
Trans-flocoumafen, 295
Transient receptor potential excitatory ion channels, 144
Trans-isoelemicin, 293
Transition metals, 264
Translationally controlled tumour protein, 53
Trans-phenyl-3-propenoic acids, 121
Treponema pallidum, 266
1-Triacontanol, 69
Triacontanyl tetracosanoate, 69
Trianthema portulacastrum, 17
Triatomine bugs, 173
Tribulus terrestris, 17
Trichogaster trichopterus, 340
Trichophyton longifusus, 209
Trichophyton mentagrophytes, 209
Trichophyton rubrum, 209
Tridec-1-ene-3,5,7,9,11-pentayne, 70
3-*cis*-11-*trans*-Trideca-1,3,11- triene-5,7,9-triyne, 70
(*Z*,*Z*,*E*)-5-(Trideca-4,7,10-trienyl)resorcinol, 92
(*Z*,*Z*,*Z*)-5-(Trideca-4,7,10-trienyl)-resorcinol, 92
(*Z*,*Z*)-5-(Trideca-4,7-dienyl)resorcinol, 92
Trigonella foenum-graecum, 18, 29
Trimethoxy-3´,4´-methylenedioxy-3,4,5´-7,9´,7´,9-diepoxylignan, 292
Triplet excited state, 260
Triplet state, 260
Triterpenes, 175
Triterpenoid, 313
Tritrophic level interactions, 137
Tropaeolum majus, 18, 24
Ion channel, 144
Trypanocidal, 175
Trypanocidal assay, 177
Trypanocidal drug design, 175, 177
Trypanocidal linear monoterpene, 173, 180, 183
Trypanocidal monoterpene hydroperoxides, 181
Trypanocidal p-cymene derivatives, 180
Trypanosoma brucei gambiense, 174
Trypanosoma brucei rhodesiense, 174
Trypanosoma cruzi, 183
Trypanosomiasis, 174, 175, 177
Trypomastigotes, 175, 177, 182
Tryptophan, 261
Tuberculosis, 46
Tumour latency, 119
Tumour necrosis factor-α (TNF-α), 56
Turnera aphrodisiaca, 18, 25, 29
Turnera diffusa, 18
Tylenchulus semipenetrans, 75
Tylenchulus semipenetrans, 103
TyrR-expressing cell system, 76

U

Ubiquination, 159
Ubiquinol, 272
Ubiquinone, 272
UDP-glycosyltransferases, 161
Ulcerative sores, 48
Umbelliferae, 49, 293, 294
Umcka ColdCare, 146
Unbound adventitious metal ions, 259
Upregulated DXS expression, 153
Urethritis, 31
Urginea indica, 18
Urginea maritima, 18, 29
Uricosuric, 48
Urinary infections, 252
Ursolic acid, 80, 175
Urtica dioica, 18, 30
Urtica urens, 18
Urticaria, 46
Usnea hirta, 18
Ustilago violacea, 209
UV irradiation, 163
Uzarigenin, 85

V

Vaccinium macrocarpon, 18, 25, 30
Vaccinium myrtillus, 18, 30
Vacuolation, 299
Vanillic acid, 87
Vanillin, 293
Vanilloid-3 (TRPV-3), 144
Vardenafil, 247
Vascular endotelial growth factor (VEGF), 60
Vascular endothelial cells, 56
Vascular endothelial growth factor, 118

Vascular permeability, 124
Vectobac, 339
Vector, of dengue, 277
Vector resistance, 278
Vectoring viruses, 67
Vegetal body identification, 233
VEGF expression, 125
VEGF mediated angiogenesis, 126
VEGF promoter, 122
VEGF-induced proliferation, 122
Verbascum densiflorum, 18
Verbascum phlomoides, 18
Verbena crinoydes, 18
Verbena officinalis, 18, 30
Verbenaceae, 292, 306, 320
Verbenone, 187
Vermifuge, 48
Verticillium dahliae, 148
Vesicant, 25
Vesicular apical formation, 299
Viburnum furcatum, 88
Vicks Vaporub, 146
Vincetoxicum hirundinaria, 18
4-Vinylphenol, 88
Viola tricolor, 18
Viomellein, 322
Viomellin, 326
Virucidals, 158
Visceral leishmaniasis, 54
Vitamin A deficiency, 164
Vitamin B-6, 30
Vitamin E, 268
Vitex rotundifolia, 320
Vitis vinifera, 18, 30
Voacanga africana, 18
Vouacapane diterpenoids, 309
Vulgarone B, 55

W

Wagner-Meerwein, 313
Westhinger, 204
Wettsteinia schusterana, 193
Withania somnifera, 18
Wormwood, 45
Wounds, 48

X

Xanthine oxidase, 263, 269
Xanthine oxidase inhibition, 124
Xanthomegnin, 322, 326
Xanthones, 175, 207, 285
Xanthonol, 322
Xenopus oocytes, 201
Xerocomus badius, 326
Xerophthalmia, 164
Xiphinema americanum, 101
Xiphinema diversicaudatum, 77
Xiphinema index, 81
Xylaria sp, 208
Xyris pterygoblephara Steud, 209, 211

Z

Zaluzanin D, 175
Zanhic, 81
Zea mays, 18, 163
4-Zimgiberaceae, 320
Zingiber officinale, 18, 30, 92
Zingiber officinale Roscoe, 320
Zingiber purpureum Roscoe, 320
Zingiberaceae, 274, 306, 308, 320